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BIOLOGIC TITRATION OF DIETHYLSTILBESTROL AGAINST ACTIVITY OF PROSTATIC CANCER: EFFECT ON SERUM ALDOLASE*

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THE concentration of a number of oxidative enzymes is reduced in various tumors. Greenstein (1) has indicated that catalase is absent in transplanted hepatoma. Other investigators (2, 3, 4) have shown amino acid oxidase, cytochrome C reductase and xanthine dehydrogenase to be present in decreased amounts in cancer. Schneider and Potter (5) reported that succinic acid dehydrogenase and cytochrome oxidase activities of rat hepatoma were only 22 to 30 per cent of those found in normal liver. There are only fragmentary data concerning the relationship of endocrine function to activity of these enzymes. Observations in this area are of particular interest as the endocrine control of cancer of the breast and prostate and certain of the leukemias becomes more apparent.

Warburg's (6) studies on tumor metabolism disclosed a quantitative difference between normal and cancer tissue which as yet remains unexplained. Malignant cells have a high and characteristic rate of aerobic glycolysis. Warburg and Christian (7) and later Sibley and Lehninger (8) reported also that one of the enzymes, aldolase, of the glycolytic mechanism appears in large quantities in the serum of tumor-bearing rats and in the serum of some patients with cancer. This observation is not of diag-

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nostic value, as only 20 per cent of all patients with cancer have increased serum aldolase levels. In addition, tumor weight must exceed 2 to 3 per cent of total body weight of the rat before a significant increase in serum aldolase is obtained.

These obvious objections to the use of serum aldolase as a diagnostic indicator for the detection of cancer are valid. Previous observations from this laboratory (9, 10) have demonstrated however, that serum aldolase concentration may be useful in determining the activity of advanced prostatic cancer. It has been shown that abnormally high concentrations of aldolase are present in the serum of 60 to 70 per cent of patients with active, advanced, untreated prostatic cancer. Following orchiectomy or administration of stilbestrol the increased serum aldolase concentration returns to normal range in 85 to 90 per cent of patients studied. It is believed, therefore, that serum aldolase is equally as effective as an activity indicator as the classically accepted biochemical indicator of advanced prostatic cancer activity, namely, serum acid phosphatase. Unfortunately, both serum aldolase and acid phosphatase are equally ineffective in measuring presence or activity of early prostatic cancer. The function of serum acid phosphatase is unknown. The function of aldolase is known, due to its high degree of substrate specificity. The relationship of aldolase to the characteristic rate of tumor glycolysis has already been briefy indicated.

This paper presents additional experimental data on two phases of the relationship of serum aldolase to the endocrine control of cancer: 1) The effect on serum aldolase of the minimal effective dose of estrogen necessary to deactivate prostatic cancer, and 2) The effect of orchiectomy on prostatic tissue aldolase.

METHODS

1. Four patients with untreated, advanced prostatic cancer were studied. Serum aldolase concentrations were determined prior to and during therapy. Treatment consisted of orchiectomy or the daily intramuscular administration of diethylstilbestrol in the doses indicated.

2. Determinations of prostate tissue aldolase content were performed on albino rats of the Sprague-Dawley strain. Five days following orchiectomy the rats were sacrificed by a blow to the head and the prostate was immediately excised, cleaned of all adventitial tissues and weighed to the nearest milligram on a Roller Smith Torsion balance. Following refrigerated homogenizing, tissue aldolase content per milligram of weight was determined in the usual manner. Sham operation was performed on control rats, using ether anesthesia and a scrotal skin incision.

3. The function of aldolase in the glycolytic mechanism of intracellular metabolism is established precisely, due to its high degree of substrate specificity. Aldolase catalyzes the reversible cleavage of fructose-1, 6-diphosphate to 1 mole each of glyceraldehyde phosphate and dihydroxyacetone phosphate. Serum aldolase was determined by the colorimetric method of Sibley and Lehninger (11).

This method for determination of aldolase is a practical procedure and the results obtained are significant for relative alterations in serum concentrations. The method presents certain pitfalls for the determination of absolute quantities of aldolase. Crystalline rabbit aldolase was extracted and recrystallized according to the procedure of Cori (12). Recovery of a known amount of aldolase by the method of Sibley and Lehninger was frequently in error to a magnitude of 15 per cent.

On occasion, serum aldolase determinations failed to reveal any of the enzyme present, while duplicate determinations of the same sample demonstrated the expected presence of a normal quantity of aldolase. The explanation for this peculiarity is unknown. As a consequence duplicate, and often quadruplicate, determinations were performed on each sample of serum or tissue.

4. Serial enzyme levels were obtained on all humans and animals in the experiment. It was necessary, therefore, to compare fluctuations in enzyme level with some other constituent of the serum. Variations in aldolase concentration per ml. of serum could conceivably be explained solely by alterations in total blood volume due to blood loss, hemodilution from intravenous fluids, or hemoconcentration due to dehydration. Serum aldolase values per ml. were compared with total protein concentration per 100 ml. in the form of an Aldolase Index.

$$\frac{\text{Serum adolase units/1 ml.}}{\text{Total protein Gm./100 ml.}} = \text{Aldolase Index.}$$

Clinically, it was seldom necessary to express aldolase as an index. On the contrary, in rats and rabbits serial serum analyses required frequent use of an aldolase index in order to obtain significant results.

All aldolase values are expressed as units which equal cubic mm. of hexose diphosphate (HDP) (calculated as if HDP were in the form of a gas) split at 38° C. per hour. Units may be converted to micromoles by dividing by 22.4. The range of normal for human serum aldolase concentration is 2.0–9.6, with a mean of 5.8 units.

5. All phosphatase units are those of King and Armstrong (13). Total protein was determined by the micro-Kjeldahl method of Am and Zuazaga (14).

RESULTS AND DISCUSSION

In 1951 it was determined (15) that the minimal effective daily dose of diethylstilbestrol necessary to deactivate metastatic cancer of the prostate was usually in the range of 0.25 to 0.50 mg. (Fig. 1). Additional data from this laboratory have indicated that the concentration of serum aldolase is apparently an indicator for activity of prostatic cancer. An extension and correlation of these investigations in 4 patients with proven, untreated, advanced, prostatic cancer is presented. The results obtained in 3 of these patients agreed essentially with the previously reported observations, namely, that 0.1 mg. of injected diethylstilbestrol administered daily for two weeks was ineffective, whereas 0.25 mg. to 0.5 mg. of the compound induced a remission of cancer activity as determined by a decrease in serum acid phosphatase and an increase in serum alkaline phosphatase. In addition, the concentrations of serum aldolase progressively decreased with increasing qualities of administered estrogen, until a maximum de-

pression was obtained with a daily dose of 0.25 to 0.5 mg. of diethylstilbestrol. In each of the 3 patients, the level of serum aldolase returned to the normal range with continued administration of this quantity of estrogen. Either increasing the dose level to 5 mg. daily, or androgen withdrawal by means of orchiectomy, did not increase deactivation of the prostatic cancer as determined by serum aldolase levels, alteration in acid and alkaline phosphatase and appearance of osseous metastases.

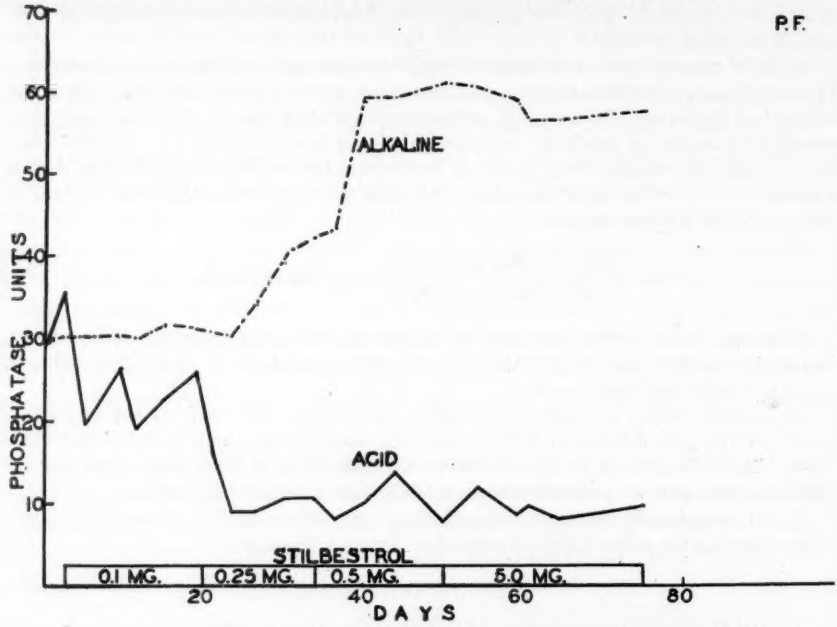

FIG. 1. Demonstration of the minimal effective daily quantity of diethylstilbestrol usually necessary to produce remission of advanced prostatic cancer.

One patient studied was an exception to this observation and is now presented in some detail in Figure 2. At no time did this patient have an elevated serum acid phosphatase level to indicate the extensive activity of his advanced prostatic cancer. The patient had diffuse osseous metastases demonstrable by x-ray examination. The serum alkaline phosphatase concentration was elevated above normal. During the pre-therapy study period, the patient was relieved of his severe spine and leg pain by administration of analgesics. As a result of adequate sleep and freedom from pain, the patient was able to consume a daily diet of 4,500 calories. A striking elevation in the level of the serum alkaline phosphatase developed. This biochemical improvement has been observed in several addi-

tional patients and presents possibilities 1) that the human organism may offer biologic resistance to the activity of prostatic cancer, or 2) the changes observed may be due to chance sampling of serum. Errors in technique were excluded. No similar alterations were observed in the concentration of serum aldolase.

As previously described, diethylstilbestrol was administered to this patient daily in amounts proceeding from ineffective to effective doses,

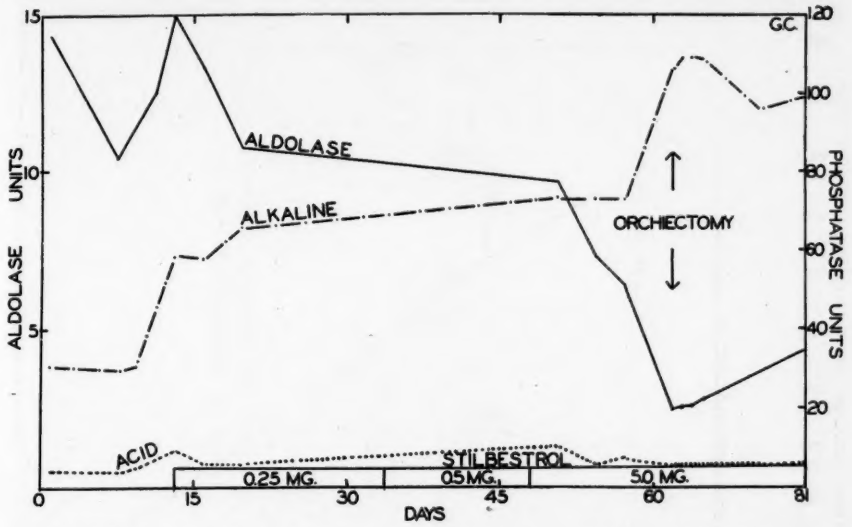

FIG. 2. Biologic titration of increasing daily intramuscular doses of diethylstilbestrol against activity of advanced prostatic cancer. Incomplete deactivation was produced with 0.5 mg., but complete remission with 5.0 mg. No additional improvement was obtained from orchiectomy. Observe normal acid phosphatase level.

each dose level being maintained for at least fourteen days. Although 0.5 mg. daily of this estrogenic compound apparently produced deactivation of the cancer (as determined by an increase in the level of serum alkaline phosphatase, a decrease toward normal in the level of serum aldolase, and subsidence of symptoms) an increase in the dosage to 5.0 mg. daily promptly resulted in additional biochemical improvement. With this quantity of estrogen the alkaline phosphatase concentration increased markedly and, simultaneously, the serum aldolase concentration decreased to the normal range. Orchiectomy failed to produce any additional biochemical improvement in the level of either serum alkaline phosphatase or aldolase.

It is conceivable that the failure of aldolase to decrease to normal levels in the serum during the 14-day period of 0.5 mg. of diethylstilbestrol

daily, was due to a normal prolonged rate of removal of the enzyme from the blood stream. To test this possibility, a dose of 7,500 units of crystalline rabbit aldolase was injected intravenously into a rabbit. Serial serum aldolase determinations revealed that an aldolase index of over 600 was obtained within thirty minutes of injection (the normal aldolase index in the rabbit is from 1 to 7). Twenty-four to twenty-eight hours later the serum concentration of the enzyme was found to be normal (Fig. 3).

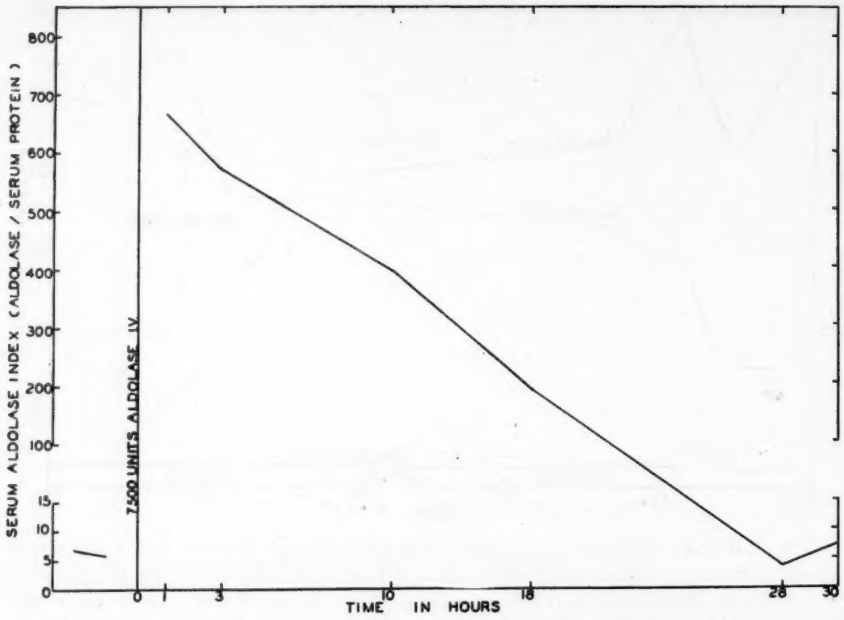

FIG. 3. The effect on the serum aldolase concentration of an intravenous injection of crystalline aldolase into the rabbit. Observe normal serum aldolase level 24–28 hours following administration. Determinations corrected against hydration.

Using the rat as a test animal, the results of Warburg and Christian agree essentially with ours, despite their failure to correct the aldolase values obtained against the state of serum hydration. It may be concluded that in patients with advanced prostatic cancer, who have increased levels of serum aldolase, the therapeutic effectiveness of diethylstilbestrol on cancer activity should be reflected by a very prompt decline in serum aldolase concentration. This condition has obtained in all patients studied.

To determine the effect of endocrine therapy on the tissue concentration of aldolase in the rat prostate, orchiectomy was performed on a group of rats. The normal rat prostate contained from 1.2 to 5.3 units of aldolase

per mg. of wet tissue. Five days following orchiectomy the prostate contained from 6.3 to 9.4 units per mg., or an increase of over 100 per cent (Fig. 4). This variation obtained, regardless of the age or nutritional status of the rat. The apparent increase observed in prostatic tissue aldolase concentration in the rat following androgen withdrawal is not reflected by a concurrent increased serum aldolase level.

The increase in the concentration of prostatic tissue aldolase subsequent to orchiectomy may not be real, or at least not as great as the foregoing

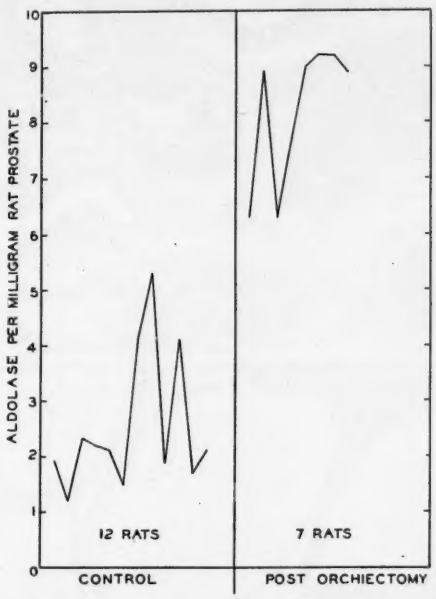

FIG. 4. Effect of orchiectomy on prostatic tissue aldolase concentration in the rat.

experiment indicates. Prior to orchiectomy the rat prostate is not a very cellular organ. The acini are numerous and large. After orchiectomy the large acinar spaces shrink and the gland becomes densely cellular. Comparison photomicrographs are presented in Figure 5. Per mg. of wet weight, there are more cells in the rat prostate following orchiectomy. In order to state definitely that androgen withdrawal produces a higher concentration of aldolase in prostatic tissue, simple measurement of the weight of prostatic tissue sampled is not sufficient. Significant and conclusive results may be obtained only by determination of the aldolase content per unit of prostatic cell prior to, and subsequent to, orchiectomy. This phase of the problem is being pursued further in humans.

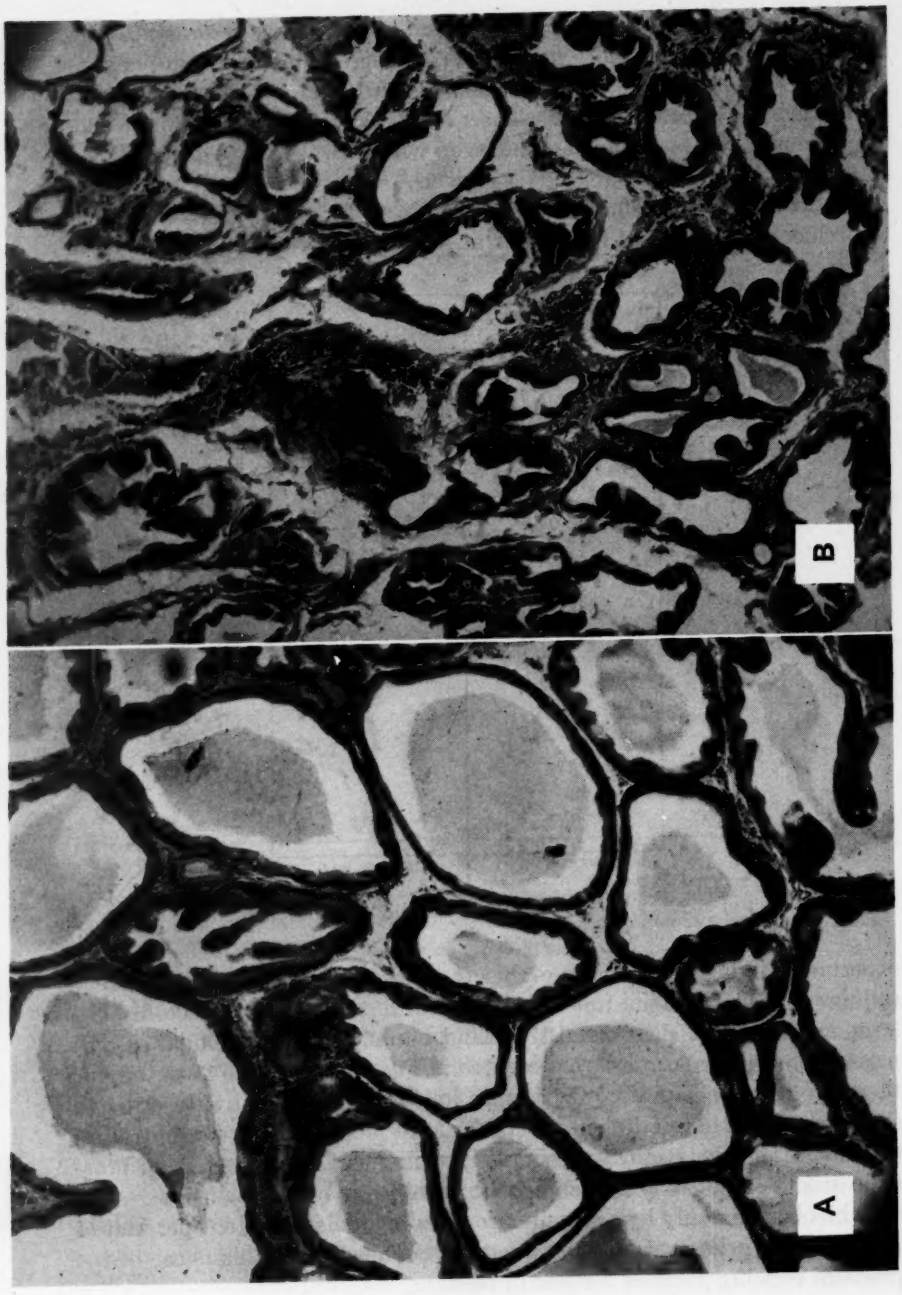

FIG. 5. A. Photomicrograph of normal rat prostatic tissue. X120. B. Comparison photomicrograph of rat prostatic tissue five days after orchiectomy. X120.

SUMMARY

Diethylstilbestrol has been administered to a series of patients with untreated advanced prostatic cancer, in dosages increasing from ineffective to effective amounts. The serum aldolase level serves equally as well as the serum acid and alkaline phosphatase levels in determining the degree of remission of cancer activity. The minimum daily effective dosage of this estrogenic substance has been previously shown to be between 0.25 and 0.5 mg. Increasing the dosage to 5.0 mg. daily, or use of orchiectomy, does not enhance the effect. An occasional patient is an exception to this observation and may require as much as 5.0 mg. of diethylstilbestrol daily to effect the maximum remission possible with hormonal therapy.

It has been demonstrated that any change in cancer activity will be reflected by a prompt fluctuation in the level of serum aldolase. The effect of orchiectomy on the concentration of prostatic tissue aldolase is discussed.

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photomicrograph of rat prostatic tissue five days after orchiectomy. X120.

DOES A LARGE INTAKE OF POTASSIUM MODIFY THE METABOLIC EFFECTS OF ACTH (CORTICOTROPIN) IN MAN?*

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INTRODUCTION

THAT the adrenal cortex plays a role in the regulation of potassium metabolism has been recognized since Baumann and Kurland (1) in 1927 observed an elevated plasma potassium level in adrenalectomized cats. Subsequently it was noted that patients with Addison's disease may develop hyperkaliemia (2) and that certain patients with Cushing's syndrome have hypokaliemia (3). Furthermore, the administration of adrenal cortical extract (4), of desoxycorticosterone acetate (5), of cortisone (6), or of adrenocorticotropic hormone (corticotropin) (7), may produce a lowered concentration of plasma potassium, an increased urinary excretion of potassium, and an intracellular potassium deficit (8). Another well established effect of hyperadrenocorticism, whether spontaneous or induced by exogenous corticotropin, is an increase in protein catabolism, which manifests itself by an increased urinary nitrogen excretion and a negative nitrogen balance (9, 10).

There are reports which suggest that these phenomena of electrolyte and organic metabolism may be interrelated, although the extent of such possible interrelationship is not clear. Kepler (11) in 1948 reported that in a patient with Cushing's syndrome a negative nitrogen balance reverted to a positive nitrogen balance when additional potassium chloride was administered. Pearson and Eliel (12) have reported the metabolic balance

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data on a patient who was given extra potassium chloride during a period of corticotropin therapy. In this case the extra potassium was started after a negative nitrogen balance had been produced by the corticotropin administration. The data show a reduction in the degree of negative nitrogen balance. The onset of this reduction coincided with the initiation of the administration of potassium chloride. However, since the urinary nitrogen declined progressively throughout the fifteen days of potassium chloride administration, regardless of whether or not corticotropin was given, the data are difficult to interpret. Whitney and Bennett (13) have reported that amounts of corticotropin which produce an increase in the urinary nitrogen of rats on a normal diet do not produce an increase in the urinary nitrogen of rats maintained on a diet high in potassium chloride. This inhibition of the catabolic effect of corticotropin is apparently specific for potassium, as a diet high in sodium chloride does not alter the effect of corticotropin on urinary nitrogen. In a comparable study, Glafkides, Bennett and George (14) have shown that a diet high in potassium chloride will inhibit the diabetes-enhancing effect of small doses of corticotropin in the partially depancreatized diabetic rat. Another well recognized effect of corticotropin is the sodium retention that occurs in man during the first few days of a corticotropin treatment period. It is self evident that the potassium-depleting, sodium-retaining, diabetogenic and catabolic effects of corticotropin are undesirable effects when corticotropin is used clinically. The following study was undertaken in an effort to determine to what extent the biologic activity of corticotropin in man might be modified by the simultaneous administration of large amounts of potassium salts.

METHODS

Seven patients were used in this study. Five of them had active generalized rheumatoid arthritis, 1 had disseminated lupus erythematosus, and 1 had hyperinsulinism.

Short-term studies. In the first phase of the study, Thorn tests (15) were carried out on Patients A. E. and J. K. Breakfast was withheld on the mornings of the tests and either a placebo of physiologic saline or 50 International Units of corticotropin was injected intramuscularly at 8 A.M. On certain days 10 grams of potassium chloride (enteric-coated) was administered in divided doses during the twelve hours preceding the injection of corticotropin. Urine was collected from 6 A.M. until 8 A.M. and again from 9 A.M. until 12 noon for uric acid and creatinine determinations. Blood was collected at 9 A.M. and again at 12 noon for circulating eosinophil counts and for serum potassium determinations.

Long-term studies. In the second phase of the study 6 of the patients were started upon a metabolic balance regimen and observations were made during treatment and control periods. A minimum of six days equilibration on the constant diet was allowed before beginning metabolic study. Corticotropin was given intramuscularly, usually

in amounts of 25 International Units each six hours. In one study cortisone was given by mouth, 125 mg. every six hours. Potassium was usually administered as the acetate, which was given by mouth in doses of 12 Gm. three times daily. This amounted to a supplemental potassium intake of 360 mEq./day in most studies. The supplemental potassium intake was started either from four to six days prior to beginning hormonal therapy, or from three to six days after beginning hormonal therapy.

While on this metabolic regimen the diet of any one patient was constant from day to day with respect to total calories, fat, carbohydrate, protein, sodium, potassium, calcium and phosphorus. At frequent intervals a duplicate of an entire day's diet was homogenized and aliquots were analyzed for nitrogen, sodium, potassium, calcium, and phosphorus. Because these analyses indicated a variation of less than 5 per cent from day to day in the composition of the diet, it was believed to be unnecessary to analyze each day's diet. In most instances in the text to follow, we present only the data for urinary excretion. This represents accurately the changes in metabolic balance, because of the constancy of the daily dietary intake and because of the very low and nearly constant level of fecal excretion. In those instances in which we present our data as a metabolic balance, the intake shown is the average intake as determined by the aforementioned analyses. Urine specimens were collected each twenty-four hours and were preserved with concentrated hydrochloric acid. Stools were collected over six-day periods and were preserved with hydrochloric acid. Carmine was used to delimit the stool collection periods. In preparation for mineral analysis, dietary and stool aliquots were dry-ashed at 400° C. for twelve hours. On alternate days, blood was drawn under fasting conditions for chemical analyses, counts of circulating eosinophils, and erythrocyte sedimentation rate determinations.

The following laboratory methods were employed. Sodium and potassium determinations were made using a Perkin-Elmer flame photometer (Model 52A) with lithium as an internal standard. Phosphorus was determined by the method of Fiske and Subba-Row (16). Nitrogen analyses were carried out employing a steam-distillation micro-Kjeldahl method. Urinary 17-ketosteroids were estimated using a modification of the method of Drekter, Callow, and Emmens (17). Blood glucose concentration was determined by the method of Nelson (18). Total circulating eosinophils were counted in a Levy chamber using a propylene glycol-phloxine diluent as recommended by Madison (19). Uric acid was determined by the method of Benedict and Franke (20). Creatinine was determined by the method of Peters (21).

Clinical examination was carried out independently by two observers each day. In evaluating the therapeutic response of the patients with arthritis to corticotropin, emphasis was placed upon objective evidence of inflammatory activity as judged by the amount of joint swelling, local tenderness and limitation of motion.

RESULTS

Clinical observations. In none of the 5 patients with rheumatoid arthritis to whom potassium acetate was administered during an extended period of corticotropin therapy was there evidence that the therapeutic effects of the corticotropin were modified. In no instance was there change in the subjective or objective clinical status when potassium administration was started or stopped during corticotropin therapy. No untoward effects were encountered in our patients during the administration of large doses of potassium salts, except nausea in some instances.

Short-term studies

Data presented in Figure 1 demonstrate that following the intramuscular injection of a single dose of 50 units of corticotropin, Patients A. E. and J. K. had an average fall of about 40 per cent in their circulating eosinophils in four hours. After twelve hours of administration of potassium

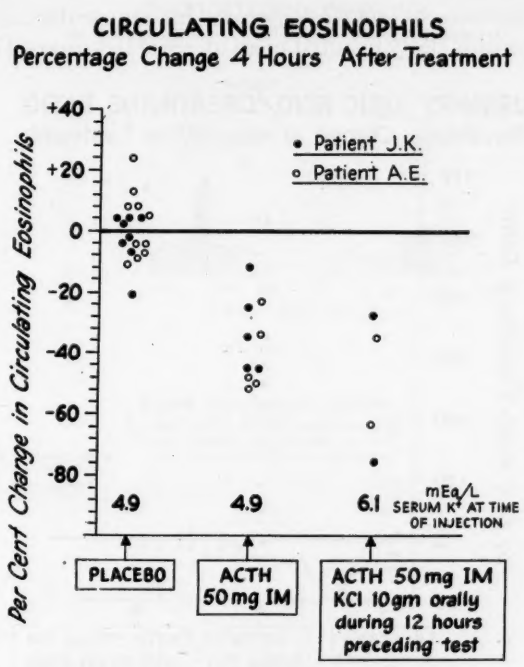

FIGURE 1

chloride in these acute tests the average fasting serum potassium level was 6.1 mEq./liter as compared to an average of 4.9 mEq./liter on control days. This indicates that good absorption of the enteric-coated potassium had occurred. However, the eosinopenic effect of corticotropin was not diminished by this preparatory potassium chloride administration. Further evidence that the eosinopenic effect of corticotropin was not diminished by potassium was obtained in the long-term studies of patients to whom potassium acetate was given during a period of corticotropin therapy. In no instance in these patients was the degree of the corticotropin-induced eosinopenia altered by either the institution or withdrawal of supplemental potassium acetate. Data from 1 patient which illustrate this point are presented in Figure 4. The data presented in Figure 2 show that

the administration of 50 units of corticotropin produced a rise in the urinary uric acid-creatinine ratio of about 60 per cent. Preparatory administration of potassium chloride also failed to prevent this effect of corticotropin.

Long-term studies

Urinary 17-ketosteroids and blood glucose: Intramuscular injection of 100 units of corticotropin daily resulted in an increase in urinary 17-ketosteroid

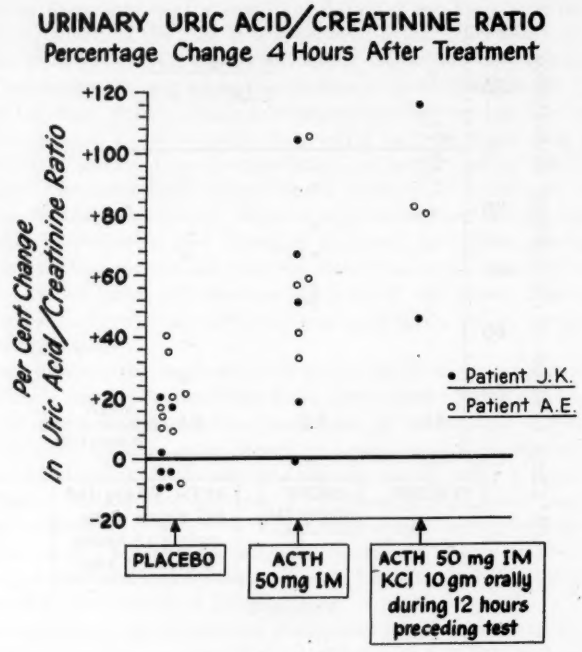

FIGURE 2

excretion. Potassium administration, whether begun before or during corticotropin therapy, did not prevent this effect of corticotropin in any of our patients. Data from 2 cases which illustrate this point are presented in Figure 3. Minor rises in the fasting blood glucose levels were observed in Patients R. D., L. G., and J. K., during corticotropin therapy, regardless of whether supplemental potassium was given.

Nitrogen balance. The daily administration of 100 units of corticotropin to our patients with active rheumatoid arthritis resulted in a stepwise increase in urinary nitrogen excretion which, in all but 1 instance, culminated in a frankly negative nitrogen balance. The same phenomenon was

observed in Patient L. G. when she received cortisone. In no instance was there evidence that this catabolic effect of corticotropin or of cortisone was inhibited by potassium. The nitrogen balance data for Patient A. E. show clearly that a negative nitrogen balance occurred when corticotropin therapy was started during the high-potassium regimen (Fig. 4). The

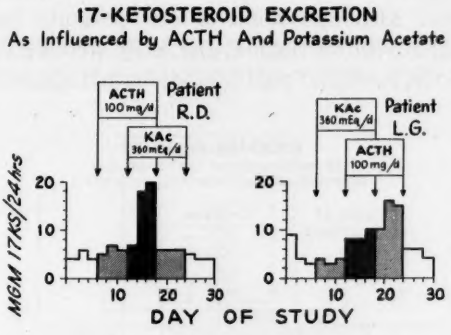

FIGURE 3

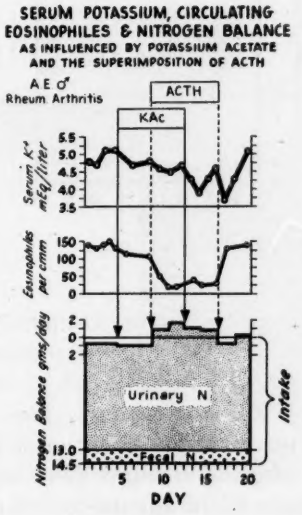

FIGURE 4

data also show that upon the cessation of potassium therapy the degree of negative nitrogen balance was not increased, as one would have expected if potassium had been inhibiting the catabolic effect of corticotropin. Comparable results were obtained when potassium was given during

cortisone therapy. The data shown in Figure 5 demonstrate that the degree of negative nitrogen balance produced by cortisone was not immediately reduced when potassium acetate therapy was started after six days of previous cortisone treatment. However, it is possible that there was a slow response and that, if the experiment had been continued for a third period, there would have been a return to nitrogen equilibrium.

Sodium balance. Although none of our patients became edematous during corticotropin administration, the most striking result encountered in this study was the profound modification by potassium salts of the effects

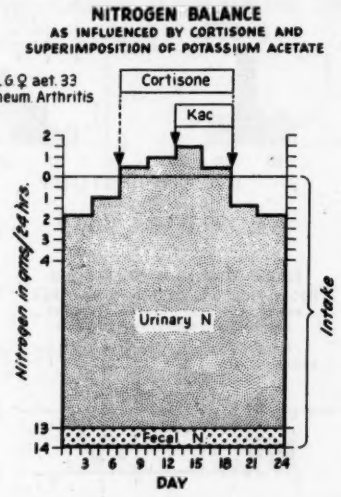

FIGURE 5

of corticotropin upon sodium excretion. Ordinarily the administration of corticotropin to man results in a marked retention of sodium during the initial days of treatment, and withdrawal of corticotropin therapy is followed by a marked diuresis of sodium. This typical pattern of the effects of corticotropin upon sodium excretion is profoundly modified by the administration of *adequate* amounts (360 mEq./day) of potassium acetate. As shown in Figure 6, the administration of corticotropin alone to Patient R. D. was accompanied by the typical retention of sodium; however, when potassium acetate was added to the therapeutic regimen six days after corticotropin therapy had been started, a prompt and striking diuresis of sodium occurred. The subsequent cessation of corticotropin therapy while potassium acetate was still being given was not followed by the sodium diuresis which ordinarily follows the withdrawal of corti-

cotropin. Still later, when potassium acetate administration was stopped, there was a phase of sodium retention.

Comparable results were obtained in Patient J. K. when, after four days of corticotropin therapy, he was given 360 mEq./day of potassium acetate while corticotropin administration continued. His urinary sodium excre-

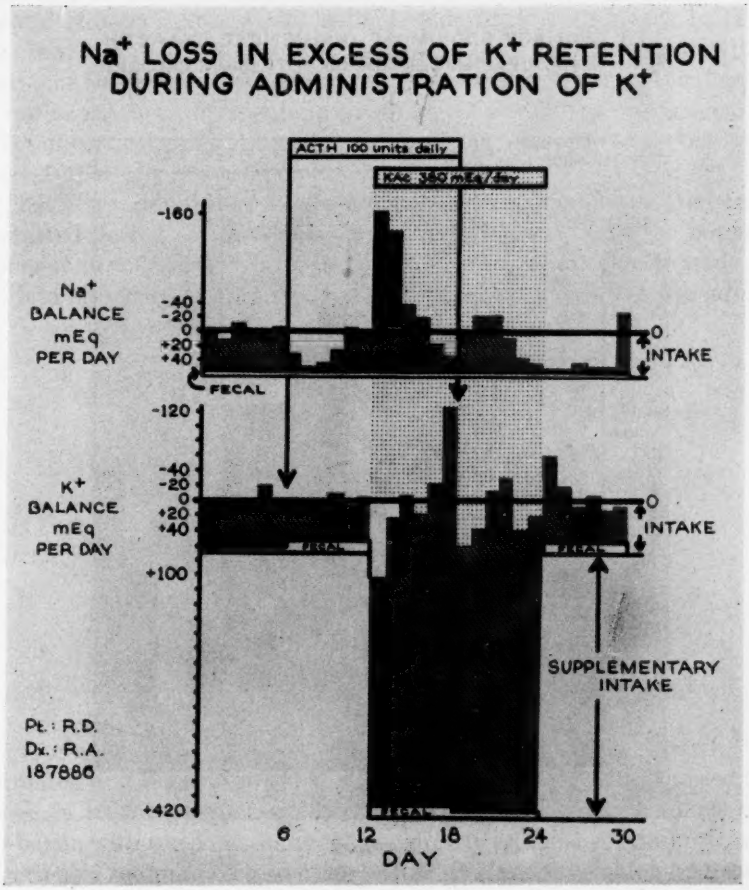

FIGURE 6

tion, which had been at the level of about 10 mEq./day, increased to about 100 mEq./day. A second experiment was carried out with Patient J. K. some months later, at which time he had been receiving corticotropin therapy continuously for approximately three months. This time he was in sodium equilibrium at the time the potassium administration was

started. When he was given 120 mEq./day of potassium acetate, his urinary sodium rose from a pre-potassium level of 100 mEq./day to a peak of 170 mEq./day. A transient phase of positive sodium balance followed the withdrawal of potassium acetate while corticotropin therapy was being continued. In Patient H. B., the administration of potassium chloride

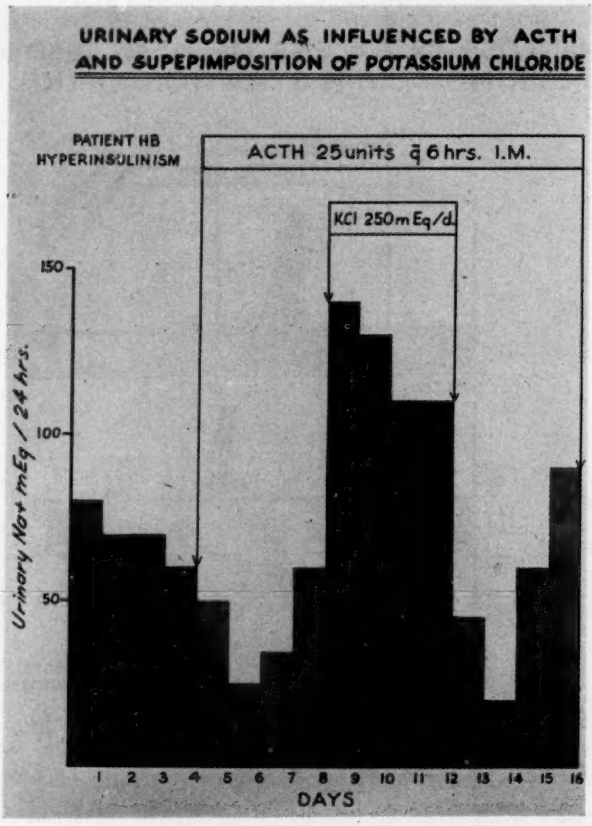

FIGURE 7

(250 mEq./day) during the middle four-day period of a twelve-day corticotropin treatment period produced moderate sodium diuresis; the sodium diuresis was both preceded and followed by a period of sodium retention (Fig. 7). The fact that sodium diuresis occurs when either potassium acetate or chloride is given seems to point to a specific role for the potassium ion.

It is appreciated that when corticotropin is given, sodium retention

does not continue indefinitely, but occurs only early in the course of corticotropin therapy. As a rule, spontaneous sodium diuresis starts near the end of the second week of treatment. It could be argued that the sodium diuresis just described which coincided with the initiation of potassium acetate administration, was coincidental, and could be attributed to the "wearing off" of, or "escape" from, the sodium-retaining effect of corticotropin. However, this would require remarkable coincidence in timing, *viz.*, four days for Patients J. K. and H. B., six days for Patient R. D., and some three months for Patient J. K. in the second study. Furthermore, in our experience, the spontaneous disappearance of the sodium-retaining effect of corticotropin is a gradual one and not the abrupt, marked sodium diuresis that occurs when potassium salts are given.

That the sodium diuresis which occurs when potassium administration is started during corticotropin therapy is actually due to the potassium rather than to "escape" from the influence of corticotropin, is made clear by data presented in Figure 8. In this experiment potassium was adminis-

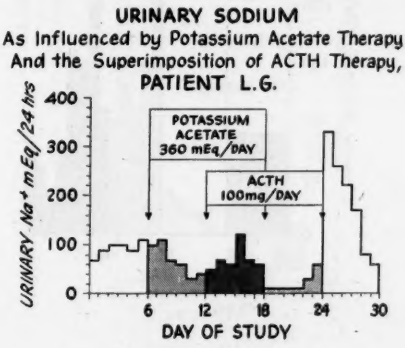

FIGURE 8

tered for a six-day period before corticotropin therapy was started. When calculated on the basis of a six-day period, sodium excretion showed little change in response to potassium acetate alone. When corticotropin therapy was begun with the potassium regimen already established, the anticipated retention of sodium failed to occur. After six days of combined potassium and corticotropin therapy, potassium alone was stopped and at this time the sodium-retaining influence of corticotropin became clearly evident. Subsequently, however, the withdrawal of corticotropin was followed by a marked sodium diuresis. Further evidence that the sodium diuretic effect of potassium salts cannot be explained by an "escape" of the adrenal cortex from the influence of corticotropin is furnished by the second study carried out with Patient L. G. (Fig. 9). In this instance

potassium acetate was given during a period of cortisone therapy, and again it produced profound sodium diuresis.

The production of sodium diuresis by the administration of potassium salts is an old observation. However, it is of interest that in Patient L. G. (Fig. 8) the administration of potassium acetate prior to corticotropin therapy did not produce sodium diuresis, although it clearly inhibited the sodium-retaining effect of corticotropin when this hormone was subsequently given while potassium administration continued. Another Patient, N. C., was maintained on a low sodium intake (6 mEq./day) during corti-

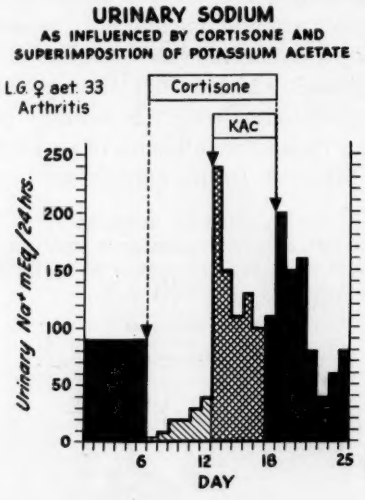

FIGURE 9

cotropin therapy. When she was given potassium acetate, starting four days after initiation of corticotropin treatment, her urinary sodium rose only to 30 mEq./day at the time of maximum sodium diuresis. In four other experiments we have given 360 mEq. of potassium per day to 2 other patients. Neither of these patients had simultaneous corticotropin treatment; in all instances sodium diuresis occurred, but in no instance did the magnitude of the sodium diuresis approach that seen in Patient R. D. (Fig. 6). These facts suggest that the absolute magnitude of sodium diuresis produced by potassium salts during corticotropin therapy is conditioned by the level of the body sodium stores, and is most marked when there has been prior sodium retention.

Potassium balance. One of the dangers of prolonged use of corticotropin is the possibility of serious depletion of body potassium and the question arises as to whether this can be prevented or corrected by supplemental

potassium. Presumably as a result of his having received corticotropin for three months prior to the second study, Patient J. K.'s serum potassium level was depressed to between 2 and 3 mEq./liter. This hypokaliemia had developed in the face of what would ordinarily be regarded as a liberal potassium intake of 120 mEq./day. Despite the fact that a state of potassium deficiency probably existed at this time, this patient was still in negative potassium balance during the first three periods of this study (Fig. 10). During the fourth and fifth periods, he was in approximate potassium equilibrium. During period six, an attempt was made to correct the potassium deficiency by administering a supplement of 120 mEq. of

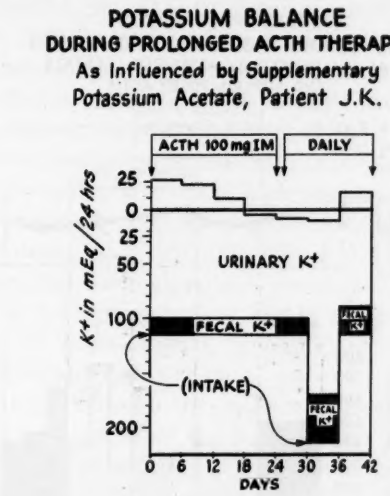

FIGURE 10

potassium acetate per day. Practically all of this added potassium was promptly excreted in the urine, and any that had been retained was lost during the negative potassium balance of the ensuing period. This shows that in this patient the prolonged use of corticotropin not only led to potassium deficiency, but that as long as corticotropin therapy was continued it was very difficult to correct this deficiency by the administration of extra potassium salts.

Phosphorus balance. In every instance in which large amounts of either potassium chloride or potassium acetate were given, regardless of whether corticotropin was also given, a striking fall in urinary phosphate was observed. This fall lasted from two to four days and in some instances was associated with a slight but definite rise of the concentration of serum inorganic phosphate (Fig. 11). In no instance was a fall in the level of

serum inorganic phosphate observed. Therefore, the decrease in urinary phosphate was due to a reduced renal clearance of phosphate. Fecal phosphorus excretion did not change, indicating that the fall in urinary phosphate reflected a transient positive phosphorus balance.

DISCUSSION

It is clear from the foregoing data that the results obtained in man when large doses of potassium salts are given together with corticotropin, are quite different from those obtained in rats. Whereas in rats potassium inhibits corticotropin-induced nitrogen wasting and corticotropin-induced

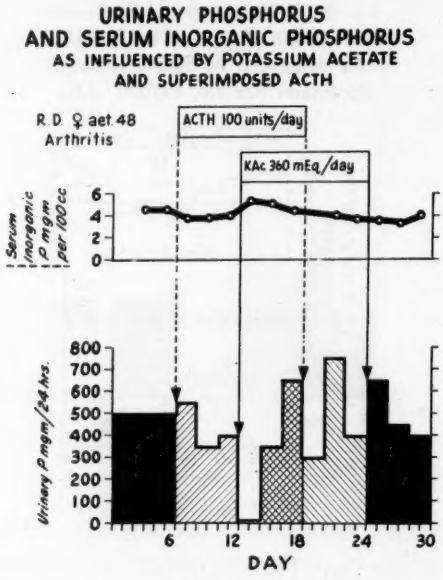

FIGURE 11

glycosuria, in man there is no evidence from our studies that potassium prevents either the catabolic effect of corticotropin or the rise in the fasting blood sugar level, which are occasioned by the administration of corticotropin. The difference in response between rats and man could be a species difference in reaction, or could be a reflection of the proportionately smaller amounts of potassium per unit of body weight which were administered to the human subjects. In these clinical experiments the maximum total potassium intake was approximately 8 mEq./Kg. per day, whereas in the comparable animal experiments the total potassium intake was approximately 30 mEq./Kg. per day. This is a level of potassium

intake which has been impossible for us to achieve in patients by the oral route. One clear-cut species difference does exist between the metabolic responses of man and of rats to corticotropin. If adrenal function is normal, the administration of corticotropin to man consistently produces sodium retention during the initial days of treatment. On the other hand, the administration of corticotropin to rats does not produce sodium retention (22).

It is of interest that when corticotropin is given to rats on a high potassium chloride diet the stimulation of the adrenal cortex by corticotropin is not inhibited (13). This is demonstrated by the fact that the adrenals of such animals increase in weight to the same extent as do the adrenals of animals on the control diet. It is further supported by the fact that there are no histologic differences found when the adrenals of the animals in the two groups are compared. These findings suggest that the inhibition of the catabolic effect of corticotropin in rats by the high potassium chloride diet is an extra-adrenal phenomenon, perhaps an inhibition of the action of the adrenocortical hormones at the cellular level. In the present clinical study there was no evidence that the high potassium intake inhibited the stimulation of the adrenal cortex by corticotropin, judging from the normal eosinopenic, uricosuric, and catabolic effects observed. However, there was clear evidence that the high potassium intake did inhibit the sodium-retaining effect of corticotropin (see Fig. 8 particularly). Therefore, in man also there is an extra-adrenal, probably renal, antagonism between potassium and adrenocortical hormones.

As pointed out previously, the sodium diuresis which occurs when potassium salts are given after initiation of corticotropin treatment is greater than that which occurs when they are given without previous corticotropin treatment, if the sodium intake is comparable (compare Figs. 6 and 8). This sodium diuresis cannot be explained entirely by the replacement of intracellular or other body sodium stores by potassium (see Fig. 6), since the amount of sodium lost is far in excess of the quantity of potassium retained.

SUMMARY

Acute experiments and long-term metabolic studies have been carried out on 7 patients, 5 of whom had rheumatoid arthritis. They received corticotropin in doses up to 100 units daily and, both separately and in combination, large amounts of supplemental potassium salts by mouth (up to 360 mEq./day of potassium acetate.) In most instances six-day balance periods were employed. No untoward symptoms occurred, except for occasional nausea.

A patient to whom corticotropin had been given for three months and

in whom marked hypokaliemia developed, did not retain extra potassium when he was given 120 mEq./day of supplemental potassium while corticotropin therapy was continued.

These large doses of potassium salts administered concurrently with corticotropin to arthritic patients failed to modify the antirheumatic effects of corticotropin. Other effects of corticotropin which were not prevented included uric acid diuresis, eosinopenia, rise in urinary nitrogen, rise in fasting blood sugar level, and rise in 17-ketosteroid excretion. On the other hand, the effects of corticotropin upon electrolyte metabolism were definitely modified. The sodium retention which occurs when corticotropin therapy is initiated was prevented or diminished by the concurrent administration of adequate amounts of potassium salts. Starting the administration of potassium salts after corticotropin therapy was underway induced marked and prompt diuresis of sodium. The marked sodium diuresis which ordinarily follows withdrawal of corticotropin therapy may not occur in patients who are receiving potassium salts concurrently. A transient (one to two days) positive phosphorus balance was produced by the administration of large doses of potassium salts.

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CONGENITAL TESTICULAR DEFICIENCY. II. DEFECTIVE SERTOLI CELL DIFFERENTIATION IN HYPOGONADISM OF SO-CALLED "OBSCURE ORIGIN"

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WE (1, 2) have recently called attention to certain forms of congenital testicular disease associated with androgen deficiency. In one type (1) there was a complete absence of spermatogonia (germinal aplasia) with preservation of Sertoli and Leydig cells. In another form (2) a rudimentary cryptorchid testis was entirely devoid of germinal, Sertoli and Leydig cells. It was composed essentially of small solid tubules containing only undifferentiated cells. The purpose of the present communication is to record still another variety of testicular dysgenesis. In this instance Leydig cells are absent, but the Sertoli cells show variable development ranging from complete absence in some tubules to full differentiation in others. At the same time, spermatogonia are preserved, although there is no evidence of germ cell maturation. Tubular fibrosis is conspicuous and could easily obscure the primarily congenital nature of the testicular lesion.

CASE REPORT

This 58-year-old native of Germany applied to the clinic because of pain in the lower back, of three months' duration. He stated that his back had been "hunched" since he was 6 years of age. Shaving was never required, He recalled penile erections approximately once a month when he was in his early twenties but these disappeared after a few years. He had had no spontaneous or induced ejaculations. Sweating, flushing, palpitations and "nervousness" had been present frequently for the previous twenty to thirty years.

His appearance is illustrated in Figure 1. There was a marked gibbus of the dorsal spine which detracted considerably from his height. The skin was soft and the cranial hair was fine and abundant despite his age. There was no recession of the temporal hair line. Facial and body hair were absent. There was a scant amount of axillary and pubic hair, the latter presenting a feminine type of escutcheon. The penis was small, and pea-sized soft scrotal testes were present. The prostate was barely palpable. There was a moderate increase of subcutaneous fat about the region of the pelvic girdle and lower abdomen, creating the appearance of wide, effeminate hips. The breasts were enlarged by a palpably increased amount of glandular tissue. The voice pitch was slightly

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elevated. Intelligence appeared to be average. The body weight was 115 pounds.

Roentgen examination of the entire spine showed considerable demineralization of all the vertebral bodies. The intervertebral space between the fifth and sixth cervical vertebral bodies was narrowed. There was marked kyphosis of the upper thoracic vertebrae. In the region of the second to the sixth thoracic vertebrae the intervertebral spaces

FIG. 1. Appearance of 58-year-old man with congenital hypogonadism, showing absence of facial and chest hair, scant pubic hair with feminine escutcheon, lack of recession of temporal hair line, small genitalia, gynecomastia and wide effeminate hips. The apparent shortening of the neck is in reality due to marked dorsal kyphosis.

could not be delineated. Partial collapse of the anterior segments of the seventh and eighth thoracic bodies resulted in wedging of these vertebrae. There was incomplete segmentation of the tenth and eleventh thoracic vertebral bodies, with degeneration and calcification of the intervertebral discs. It could not be definitely ascertained whether the vertebral nonsegmentation was congenital or merely an artefact, due to technical difficulties in visualizing the distorted intervertebral spaces. Considerable hypertrophic changes were present about most of the vertebrae throughout the length of the spinal column. Roentgen examination of the skull showed no osteoporosis or abnormality of the sella turcica.

The concentration of serum calcium was 11.4, phosphorus 1.7 and cholesterol 480 mg. per 100 cc. The alkaline phosphatase level of the blood was 5 King-Armstrong units. The basal metabolic rate was +2 per cent. A blood count and urinalysis gave normal results.

Urinary gonadotropins were assayed by the method of Zondek (3) as modified by Klinefelter, Albright and Griswold (4). In our laboratory the normal range is between 5 and 50 mouse uterine units (m.u.u.) per twenty-four hours. The patient's urine contained an excessive amount of gonadotropin, a positive reaction being obtained at 150 mouse uterine units per twenty-four hours. This assay was made on a one-fourth aliquot of a

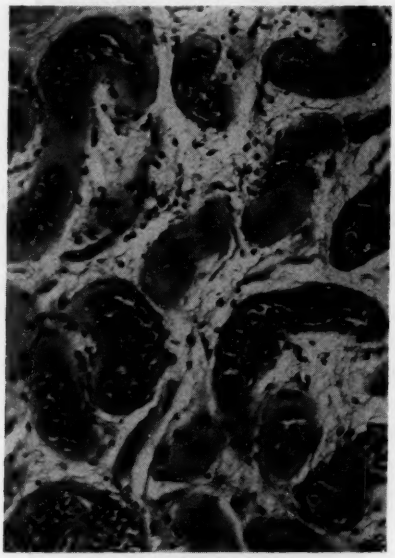

FIG. 2. Histologic section from testicular biopsy specimen (low power), showing marked tubular fibrosis resulting in complete sclerotic obliteration of some tubules. The compact epithelial contents of the unobliterated tubules are composed of undifferentiated cells and spermatogonia. The tubules lack lumens. The intertubular tissue is loose and fairly cellular but contains no Leydig cells (H. & E. ×75).

24-hour icebox-stored collection. The aqueous extract of the alcohol precipitate was tested at three dosage levels in 10 immature female mice, using 5 other animals as controls. Positive results (indicated by 100–150 per cent increase of uterine weight over that of controls) were obtained in 4 out of 4 mice at the 25 m.u.u. level, in 3 out of 3 at the 75 m.u.u. level and in 3 out of 3 at the 150 m.u.u. level. The response at higher levels was not determined.

Testicular biopsy showed varying degrees of tubular fibrosis (Figs. 2 and 3). Many tubules were small and completely sclerotic and hyalinized. Other, somewhat larger tubules exhibited thickening and fibrosis of the basement membrane and tunica propria. These lacked lumens and contained two principal types of cells—undifferentiated cells and spermatogonia—compactly distributed throughout the tubule. The majority of the cells were of the undifferentiated variety, with round or ovoid nuclei containing fine and coarse chromatin granules enclosed by a distinct nuclear membrane. Their cytoplasm

was syncytium-like and lacked a definite outline. Spermatogonia were present in fair numbers usually lying against the thickened basement membrane. Well differentiated Sertoli cells were absent in those tubules showing moderate or marked fibrosis, but an occasional partly differentiated Sertoli cell could be identified by its faint nucleolus and elongated cytoplasmic mass.

FIG. 3. Histologic section from testicular biopsy specimen (high power), showing a fair number of spermatogonia lying against the thickened basement membrane. The highly chromatic nuclei are surrounded by large zones of clear cytoplasm. The nuclei of the undifferentiated cells are smaller and denser. Their cytoplasm forms a syncytium-like mass. The lamina propria is markedly thickened (H. & E. ×500).

An occasional, partly fibrosed tubule contained a central small round homogeneous eosinophilic mass. This was embedded in the cytoplasmic syncytium, with which it appeared to merge. Its diameter was three to four times that of adjacent nuclei. The significance of this finding is not clear but attention is called to its similarity to the central colloid-like substance described by Sniffen (5) in occasional tubules of the normal testis during the first five or six years of life.

Near the surface of the gland adjacent to the tunica vaginalis several larger tubules with little or no fibrosis showed fully differentiated Sertoli cells and spermatogonia but no undifferentiated cells.

The intertubular connective tissue was fairly loose and cellular but contained no Leydig cells. There was no interstitial fibrosis or inflammatory infiltration. Thickening and hyalinization of the arteriolar wall was occasionally noted.

DISCUSSION

The introduction of testicular biopsy and urinary gonadotropin assay has provided clinical endocrinology with important tools in the diagnostic

evaluation of hypogonadism. Primary and secondary testicular deficiency are thus readily distinguished and various morphologic substrates of the former have been characterized. Despite these advances there are frequent instances of hypogonadism which remain etiologically obscure. The possibility of congenital factors in such patients is ordinarily included in general nosologic discussions (6–9) but criteria for their recognition have been inadequately elucidated.

Close attention to histopathologic detail in conjunction with a systematic search for associated developmental defects elsewhere in the body may lead to the identification of congenital testicular defects which otherwise might escape detection. Familiarity with the developmental histology of the normal testis is a necessary prerequisite for the recognition of congenital aberrations. The histologic development of the normal testis has been reviewed elsewhere (5) and only the following points need be stressed here:

1. From fetal life until puberty, the testicular tubules contain spermatogonia and undifferentiated cells. Interstitial cells of Leydig are prominent antenatally and disappear within a few months after birth.
2. The advent of adolescence is characterized by the initiation of spermatogenesis, full differentiation of Sertoli cells from the undifferentiated cells, and the reappearance of Leydig cells.

The significant testicular histopathologic findings in the patient described in the present report are summarized as follows:

1. Absence of interstitial cells of Leydig.
2. Defective Sertoli cell differentiation.
3. Conspicuous tubular fibrosis.

The absence of Leydig cells is consistent with the degree of hypogonadism but sheds no light on the pathogenesis of the lesion. It is impossible to ascertain whether Leydig cells had ever been present. However, the presence of characteristic symptoms of the male climacteric suggests the earlier existence of functioning cells.

Imperfect Sertoli cell differentiation provides the essential clue to the recognition of the congenital nature of the testicular lesion. The majority of tubules contained only undifferentiated cells and spermatogonia. It will be recalled that this pattern represents the cellular composition of the normal immature testicular tubule. The presence of such tubules in an adult with primary hypogonadism is indicative of a developmental defect. Of interest is the fact that a small number of more mature tubules could be found in certain portions of the section. In these, most of the undifferentiated cells were transformed into well differentiated Sertoli cells.

The presence of varying degrees of tubular immaturity indicates that defective gonadogenesis in this case was not uniform.

Since Sertoli cells are not normally present at birth there may be an objection to alluding to their continued absence in adult life as a congenital defect. By definition of terms, this objection is valid but of only technical importance. It is a reasonable assumption that such failure of differentiation into Sertoli cells is due to an inherent incompetence which was present at birth and is therefore congenital. The presence of a presumptively congenital spinal anomaly lends support to the concept of a congenital defect in the testis as well. Multiple developmental defects are not uncommon and may simultaneously involve extragenital and gonadal structures.

Superficially, the most striking histologic alteration was the extensive tubular fibrosis. This could have easily obscured the fundamental nature of the testicular lesion. In this event this patient would have been relegated to the all-too-frequent category of hypogonadism "of obscure origin."

The fibrotic process was limited to the walls of the tubules and did not involve the interstitium. It was most marked about those tubules which showed the greatest lack of Sertoli cell differentiation. These were the tubules that contained only spermatogonia and undifferentiated cells. By contrast, the more mature tubules containing spermatogonia and well differentiated Sertoli cells showed little or no fibrosis. The preservation of spermatogonia and the absence of evidence of Sertoli cell degeneration strongly suggest that the tubular fibrosis was not a cause of the cellular changes. If tubular fibrosis were responsible for the Sertoli cell findings, one would expect the prior disappearance of the more vulnerable spermatogonia in the manner characteristic of focal tubular atrophy of sclerotic origin. It appears likely, in the present case, that tubular sclerosis was secondary and occurred in direct proportion to the degree of immaturity. In other words, the greater the developmental defect the greater the tendency to fibrosis. The occasional finding of arteriolar sclerosis may be etiologically related to the tubular fibrosis. However, the over-all intensity of the latter was probably related to the patient's age. A similar relationship (unassociated with vascular alterations) was observed by us (1) previously in 2 brothers with germinal aplasia, another variety of testicular dysgenesis. The patient, who was ten years older than his brother, showed much more extensive tubular fibrosis.

Accurate interpretation of the testicular histopathologic picture in this patient depends firstly on the fact that urinary gonadotropins were present in increased amounts. Once established, this observation excludes the possibility that the testicular lesion is due to inadequate adenohypophyseal gonadotropin secretion. The latter condition may result in a

histopathologic picture superficially resembling that present in this patient. Secondly, the recognition of tubular immaturity as distinct from degeneration is vital for correct appraisal of the fundamental nature of the primary defect. It is not difficult to identify undifferentiated cells in the seminiferous tubules and to differentiate them from atrophic or degenerated Sertoli cells. In this manner, the lesion can be distinguished from the effects of simple tubular fibrosis or of the Klinefelter-Reifenstein-Albright syndrome. The latter condition is further rendered unlikely in this patient by the total absence of Leydig cells.

SUMMARY

A 58-year-old man with primary hypogonadism is presented. The testicular histopathologic picture was that of a developmental defect characterized by imperfect Sertoli cell differentiation. More striking than the cellular alterations was the tubular fibrosis which grossly dominated the lesion. Its intensity was sufficient to obscure the primarily congenital nature of the testicular disease, if the latter possibility were not kept in mind. Leydig cells were absent, but this condition could not be ascribed definitely to a congenital cause.

Acknowledgment

We are indebted to Dr. Earl T. Engle for his aid in the interpretation of the testicular biopsy sections.

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INCREASED URINARY ESTROGEN EXCRETION ASSOCIATED WITH ADRENAL TUMORS: REPORT OF FOUR CASES*

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INTRODUCTION

THE small number of reported cases of adrenal tumor accompanied by increased urinary excretion of estrogens suggests that this association is uncommon. Four additional cases are described here. Two of these patients also showed greatly increased excretions of neutral 17-ketosteroids and of dehydroisoandrosterone.

METHODS

The determinations of urinary hormone excretion were made by the routine clinical methods used in our laboratory. Steroids other than corticoids were extracted with carbon tetrachloride after acid hydrolysis. The technic for total neutral 17-ketosteroids was a modification of that described by Holtorff and Koch (1). Dehydroisoandrosterone was determined by the method of Allen, Hayward, and Pinto (2). In the 2 patients with increased neutral 17-ketosteroid excretions, the "phenolic fraction" obtained by extraction with 1N KOH was separated from the carbon tetrachloride extract and was used for bioassay of estrogens. This technic separates the true estrogens from the androgens and is necessary, since dehydroisoandrosterone and certain other androgens have been shown to have a vaginal cornifying effect(3). Bioassay for estrogens was performed at selected levels in castrated mice, using the vaginal smear technic. Corticoids were determined by a modification of the technic described by Heard, Sobel and Venning (4), and involved extraction with chloroform from acidified urine for one hour with an automatic shaker. Gonadotropins

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were extracted and assayed by a method slightly modified from that of Klinefelter, Albright and Griswold (5). Assay values for normal individuals are given in Table 1.

TABLE 1. HORMONE ASSAYS IN 4 PATIENTS WITH ADRENAL TUMOR

Date	Gonadotropins, m.u.*/24 hrs.		Estrogens, m.u.*/24 hrs.		Neutral 17-ketosteroids, mg./24 hrs.	Corticoids, mg./24 hrs.	Remarks
			Case 1—male, aged 30 years				
1950:							
Sept. 8	>24	<48	>80		11.3	4.0	
Sept. 15			>4000	<8000	12.4		Adrenal tumor removed Sept. 20, 1950.
Sept. 29	>24	<48		<27	9.7		
Oct. 5			>27	<40	11.8		
Dec. 27			>27	<44	7.8		
Dec. 29	>8	<24			14.9		
1951:							
Sept. 14							
1952:							
Jan. 15	>8	<24	>27	<44	9.6		
Mar. 3		<8	>27	<44	11.5		
			Case 2—male, aged 58 years				
1950:							
Nov. 23			>460	<660	5.8		
1951:							
Apr. 22	>24	<48	>13	<27	6.9		Adrenal tumor removed Nov. 21, 1950.
			Case 3—female, aged 48 years				
1950:							
Jan. 31					80.0		Dehydroisoandrosterone, 25 mg./24 hrs.
Feb. 1					85.9		Adrenal tumor removed Feb. 8, 1950.
Feb. 20					3.1		
Mar. 26					8.6		
May 21			>1000	<2000	2.4		Oral estrogen, 1.25–2.50 mg./day, from Apr. 21 to June 24, 1950.
Aug. 2					33.5		
Aug. 23					57.5		
Sept. 7					72.0		
Sept. 14					44.8		
Sept. 22	>48	<96			143		
Sept. 23			>400	<660	170		
Sept. 24 to Oct. 10			>400	<660	162 to 320	11.7	Dehydroisoandrosterone, 27 mg./24 hrs.
Oct. 11 to Oct. 15					90 to 173	12.8 to 8.2	Adrenal cortical extract, i.m., 10 cc. daily for 3 days; 25 cc. on the fourth day.
Oct. 16			approx.	1600	199	10.3	
			Case 4—male, aged 20 years				
1950:							
Nov. 20		>8	approx.	6600	105		
Dec. 21	>4	<8	approx.	4800	130	4.4	Dehydroisoandrosterone, 54 mg./24 hrs.
1951:							
Jan. 13					154		
Jan. 27			>4000	<8000	164		
Jan. 28					155	3.4	
Feb. 19				<16	3.5	3.0	Adrenal tumor removed Feb. 10, 1951. No postop. cortisone for 3 days.
Apr. 25					6.4	1.9	Cortisone, 25 mg. by mouth per day.
Apr. 26					10.4		Cortisone, 25 mg. by mouth per day.
May 21					11.8	3.4	Cortisone stopped May 2, 1951.
Oct. 14			>27	<44	8.6	3.4	
1952:							
Mar. 1 Mar. 2	>24	<48	>13	<27	10.0	3.5	
			Approximate limits of normal values				
Men	>8	<48	>13	<44	8–25	2–5	Dehydroisoandrosterone <2 mg. per 24 hrs.
Women	>8	<86	>13	<133	8–15	2–4	

* m.u. = mouse units.

CASE REPORTS

Case 1. J. S. was a young male with hypertension, gynecomastia, testicular atrophy and a tumor of the right adrenal cortex associated with greatly increased urinary excretion of estrogens but normal excretion of neutral 17-ketosteroids and gonadotropins.

The patient, a teacher of mathematics, aged 30 years, was admitted to the hospital on September 5, 1950 with a chief complaint of attacks of pain beneath the right scapula for the past few months. These were associated with effort and relieved by rest or by local pressure. He had also noticed a gradual increase in the size of his breasts for the preceding three or four years and a more rapid increase in size during the last few months. There was a doubtful history of decrease in libido. No change in voice or hair growth had been noted. Muscular weakness and occasional nausea had occurred intermittently. Hypertension had been discovered six years earlier. The systolic blood pressure varied from 140 to 200, and the diastolic from 100 to 135 mm. of Hg. Early retinal arteriolar sclerosis was present, and the heart was questionably enlarged by orthodiagram. The electrocardiographic changes were suggestive of left ventricular hypertrophy. There was a soft apical systolic murmur. Renal function was normal. The response to Benzodioxane was negative, but the cold pressor test resulted in marked increase in blood pressure. There was bilateral, slightly tender and somewhat nodular, enlargement of both breasts, greater on the left (Fig. 1a). The testes were slightly soft and somewhat smaller than normal (see pathologic report). The penis, prostate, hair growth and distribution, and body configuration (except for his breasts) were those of a normal male. An intravenous urogram showed normally functioning kidneys with a large spherical mass in the right suprarenal area displacing the kidney downward. The total eosinophil count was 212, with a fall to 78 per cu. mm. four hours after subcutaneous injection of 0.7 ml. of 1:1000 epinephrine. The total serum cholesterol concentration was 358 and 375 mg./100 ml., with a normal percentage of esters. The basal metabolic rate was −7 per cent. The results of liver function tests, including total serum bilirubin and bromsulphalein retention, were normal. Other tests which resulted in negative or normal findings included oral glucose tolerance, blood urea nitrogen, serum albumin and globulin, blood lipids, routine blood counts and urinalyses, and serologic tests for syphilis. Roentgenograms of the skull and chest showed nothing abnormal. Urinary hormone assays are shown in Table 1.

On September 20, 1950 an operation was performed by one of us (H. Z.) through a a right subcostal incision and an encapsulated adrenal tumor removed (see pathologic report). The day after operation the patient remarked that the tension in his breasts was reduced. The postoperative course was uneventful.

The patient was readmitted to the hospital for follow-up study on December 28, 1950, three months postoperatively. He reported that the symptoms present before operation had disappeared almost entirely and his only complaints consisted of occasional rectal bleeding, dull cramp-like pain in both lower abdominal quadrants on walking, and occasional dull aching in the left scapula and the left side of the neck. There had been no change in his libido. On physical examination the only alteration of significance was a decrease in his hypertension, the systolic pressure varying from 120 to 162 and the diastolic from 80 to 110 mm. of Hg. There was no apparent change in the gynecomastia or in the size and consistency of the testes. The retinal arteries still showed moderate attenuation. The source of the rectal bleeding was not discovered by roentgen or by direct endoscopic examination. No evidence of recurrence of the adrenal cortical tumor was found.

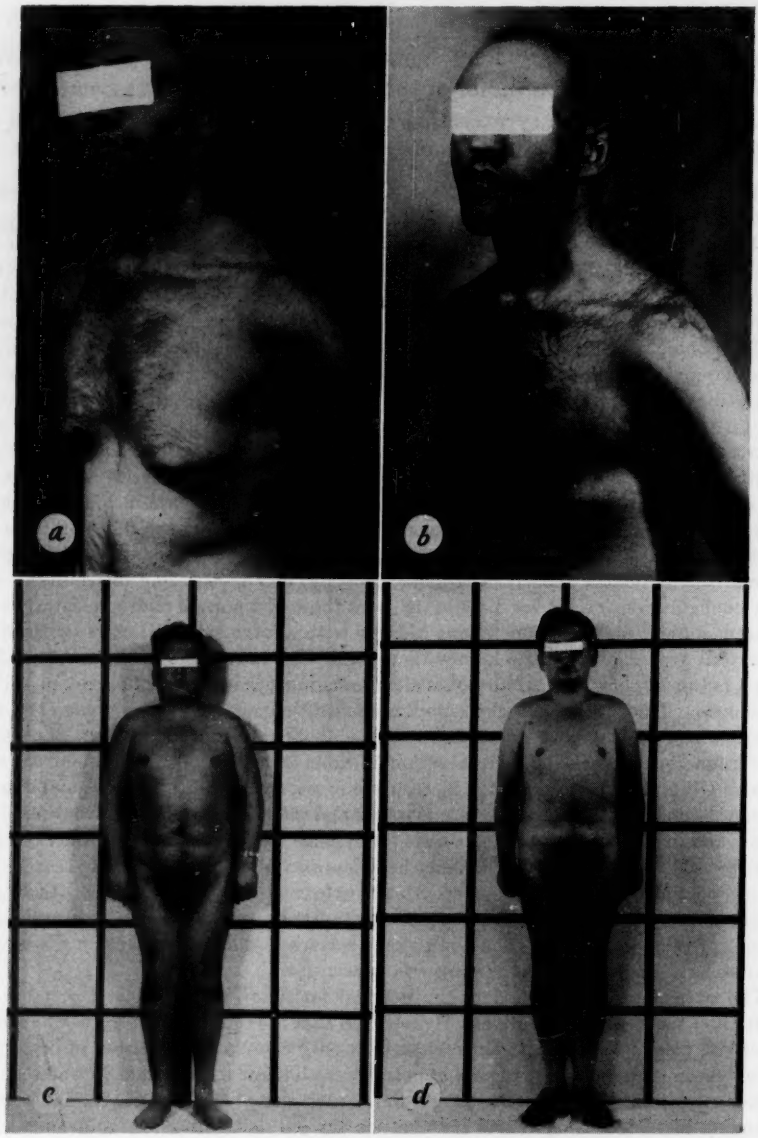

FIG. 1. (a) J.S. (Case 1) prior to operation, showing marked gynecomastia without other evidence of feminization. (b) J.S. (Case 1) seventeen months following removal of adrenal cortical tumor. The firm nodular mammary tissue previously palpable could no longer be outlined, although his breasts were still larger than normal. (c) I.S. (Case 4) prior to operation, showing body configuration, ample body hair, and small genitalia. (d) I.S. (Case 4) seven months after removal of an adrenal cortical tumor. Note change in body configuration.

The patient was again studied in the hospital from September 14 to September 16, 1951, one year after operation. His only complaints included occasional slight headache and vertigo, and frequent cough with mucoid expectoration, sometimes preceded by slight nausea. There had been no change in libido but he had noted that his breasts were definitely smaller and he had gained five pounds (2.3 Kg.). Examination showed a further decrease in blood pressure, which also showed less fluctuation than previously and averaged 130/82 mm. of Hg. The firm breast tissue had disappeared and the areolae were less darkly pigmented. There was no change in the testes. An intravenous urogram revealed no abnormality. Roentgenograms showed the skull and chest to be normal.

On March 4, 1952, a year and a half postoperatively, the areola of the breasts still showed a small area of increased pigmentation. The breasts were still somewhat large but no firm tissue was palpable (Fig. 1b). His blood pressure was 142/98 mm. of Hg. There was no evidence of recurrence of the tumor.

Pathologic report. The resected specimen (82218) consisted of an encapsulated spherical mass of orange-yellow tissue measuring 8.5×7.0×4.5 cm. and weighing 175 Gm. Septa divided the tumor into coarse nodules. These consisted of large islets of polyhedral cells having very pleomorphic nuclei and abundant eosin-staining cytoplasm. Mitoses were numerous. Stroma was scanty but the tumor cells were in intimate relationship, with numerous vascular channels. No blood vessel or capsular invasion was evident. Foci of necrosis were present.

A preoperative biopsy (82336) specimen of the left breast showed dense, acellular hyalin connective tissue in which there were tortuous ducts that exhibited occasional budding. Hyperplasia of the ductal epithelium was present and there was an occasional lymphocyte in the stroma. A testicular biopsy specimen, obtained at the same time, disclosed scattered tubules in which there was no evidence of spermatogenesis. The lamina propria was thickened, and Leydig cells were scarce. The diagnoses were adenoma of the adrenal cortex, hypertrophy of the male breast and atrophy of the testis.

Comment. The principal features associated with this adrenal cortical tumor in a young adult male included gynecomastia, hypertension, testicular atrophy and a marked increase in urinary estrogen excretion, without alteration in hair growth or distribution. The urinary estrogen values had returned to normal nine days after removal of the tumor (Table 1), but the gynecomastia persisted for more than three months. Eighteen months after removal of the tumor the gynecomastia had disappeared and hypertension had subsided considerably.

Case 2. E. M. was a middle-aged white male, with a large tumor of uncertain origin in the left adrenal associated with hypertension, gynecomastia and increased urinary estrogens. Six months after removal of the tumor, he succumbed to widespread metastases from an apparently unrelated malignant neurilemmoma. Urinary estrogen values returned to normal after removal of the tumor.

The patient, a merchant, aged 58, was first admitted to the private hospital service of Dr. W. W. Dyer[1] on November 13, 1950, complaining of nocturia, weight loss, and fatigue. During the preceding year, he had suffered a series of superficial, subcutaneous infections and had lost about 40 pounds (18.2 Kg.) despite a fair appetite and efforts to increase his caloric intake. He had also noted increasing fatigue, nocturnal frontal head-

[1] We are grateful to Dr. Dyer for permission to report this case.

dyspnea, palpitation, burning precordial pain, and edema of the ankles and periorbital areas. She had also discovered a mass in her abdomen. Her last menstrual period was in January 1949. She had lost 40 pounds (18.2 Kg.) in two years. The patient exhibited marked hirsutism, with facial hair sufficient to require shaving once or twice daily. She also showed atrophy of the breasts, facial acne, florid skin, increased sweating, tremor, and hypertrophy of the clitoris. Systolic blood pressure ranged from 176 to 128 and diastolic from 106 to 86 mm. of Hg. Deep in the left upper quadrant of the abdomen there was a large hard mass which moved with respiration.

Hormone assays are shown in Table 1. Other special examinations on the patient's first admission revealed the following: 1) Frequent hypoglycemia (fasting blood sugar values ranging from 25 to 71 mg./100 ml.). Results of a glucose tolerance test were as follows: fasting, 25; ½ hour, 104; 1 hour, 163; 2 hours, 105; 3 hours, 63; 4 hours, 33; and 5 hours, 38 mg./100 ml. 2) Electrolyte changes (serum sodium, 145; potassium, 2.8; and carbon dioxide, 32.9 mEq. per liter; serum inorganic phosphorus, 2.1 mg./100 ml.). 3) Elevated basal metabolism (+57 and +60 per cent) with a subnormal uptake of I^{131} by the thyroid (7.6 per cent at 24 hours). 4) A low blood eosinophil count (20 per cu. mm.). 5) Normal or negative values for hemoglobin, red blood cells, leukocytes, blood urea nitrogen, total serum protein, alkaline phosphatase, thymol turbidity, cephalin-cholesterol flocculation, serum calcium, chlorides and cholesterol, and serologic tests for syphilis. 6) A few red blood cells and a trace of albumin in the urine. 7) X-ray evidence of a large soft tissue mass depressing the left kidney and elevating the left dome of the diaphragm, and of an enlarged pituitary fossa measuring 13×13 mm. 8) Normal visual fields.

On February 9, 1950 an adrenal tumor and the left kidney to which it was adherent were removed through a left subcostal incision by Dr. John M. Howard. An adjacent metastatic nodule was also excised (see pathologic report). The immediate postoperative course was uneventful. Postoperatively the patient received large amounts of adrenal cortical extract.

The patient's strength improved and there was marked loss of body hair and decreased growth of facial hair until six months after the operation. During this period severe hot flashes had become more troublesome and, for these, small amounts of estrogen were given. Vaginal bleeding of eleven days' duration was experienced in June. Estrogens were stopped during the flow and not given again thereafter.

In mid-August, six months postoperatively, nocturia appeared again and the patient noted increased need for shaving. There was also an increase in the size of the liver and a rise in the urinary excretion of neutral 17-ketosteroids (Table 1). She was hospitalized three times in the next ten weeks. The patient's condition grew progressively worse. Hirsutism and acne became quite marked. She had enormous nodular hepatic enlargement, bilateral swelling of the parotid glands, and marked generalized edema, with normal blood pressure. There were frequent attacks of hypoglycemia. In addition, she showed azotemia, hyperphosphatemia, albuminuria, convulsions, and fever to 105° F. shortly before her death on November 10, 1950, nine months after operation.

During the last two months of her course, the effect of adrenal cortical extract in oil[2] upon the urinary excretion of neutral 17-ketosteroids was tested (Table 1). No inhibitory effect was found. Irradiation of the pituitary was then commenced in order to test the possibility that the widespread, but probably functioning, metastases might be under some measure of pituitary control. It was impossible to give sufficient dosage to test

[2] Lipo-Adrenal Cortex (R) supplied by the Upjohn Company through the courtesy of Dr. H. F. Hailman.

this adequately. A primary abnormality of the pituitary seemed possible, since there was roentgen evidence of enlargement of the sella turcica. Values for serum sodium, potassium, chloride, carbon dioxide and blood sugar showed marked variability during the last few months of the patient's life.

On September 12, 1950, the glucose tolerance test showed the following values for blood sugar: fasting, 61; ½ hour, 161; 1 hour, 246; and 2 hours, 183 mg./100 ml. A leukocytosis persisted, becoming more marked prior to her death. Roentgen evidence of pulmonary metastases was found, but there was no evidence of metastases in the humeri, femora, skull or spine. An intravenous urogram showed a functioning right kidney displaced downward by the large hepatic mass. The heart was not enlarged, as determined by orthodiagram.

Pathologic Report. The surgical specimen (77176) consisted of a smooth rubbery multicolored mass weighing 2,190 Gm., which included the left kidney and the adrenal tumor. Microscopically the tumor was very vascular. It was composed of sheets, cords, and bands of pleomorphic epithelial cells. Some cells had abundant vacuolated cytoplasm but in others the cytoplasm was scanty and stained densely with eosin. Nuclear pleomorphism was marked. Foci of necrosis were numerous. Tumor cells obviously invaded the fibrous tissue capsule and grew within vascular channels. The diagnosis was carcinoma of the left adrenal cortex.

At necropsy (50-305) morphologically similar tumor tissue was found in the thyroid, lungs, liver and retroperitoneal lymph nodes. The breasts and ovaries were atrophic. The right adrenal gland weighed 7 Gm. and was unremarkable both grossly and microscopically. The remaining kidney showed evidence of acute pyelonephritis. Permission to examine the cranial contents was refused.

Comment. There are several features of particular interest in this case of a woman with a virilizing syndrome due to adrenal cortical carcinoma. These features are:

1. Atrophy of the breasts, despite large amounts of estrogens in her urine. This may possibly be attributed to the inhibitory effect of circulating androgens (as indicated by the large amounts of neutral 17-ketosteroids and dehydroisoandrosterone in her urine).
2. The frequent episodes of hypoglycemia — of special interest, in view of the elevated excretion of reducing corticoids. In addition to other possibilities, they suggest the presence of a substance antagonistic to the glucogenic effect of cortisone-like steroids. Two cases of unexplained hypoglycemia in association with adrenal tumors have been reported by others (6, 7).
3. Moderate disturbance in electrolyte pattern and balance, similar to that exhibited by patients with Cushing's syndrome. This aspect of the case has been presented by Elkinton, Squires and Crosley (8).
4. The marked increase in metabolic rate, in association with the low I^{131} uptake by the thyroid. This is difficult to explain. Further data concerning the type of corticoids secreted by the tumor would be of interest, because of the known augmenting effect of cortisone on the metabolic rate and its depressing effect upon iodine uptake by the thyroid (9).
5. The failure of adrenal cortical extract to influence the excretion of neutral 17-ketosteroids. This was expected, since it seems probable that the adrenal cortical carcinoma was no longer under pituitary control and furthermore, the dose was not large. Wilkins (10) has demonstrated inhibition of netural 17-ketosteroid excretion with cortisone in patients with the adrenogenital syndrome. Jailer (11), however,

using large doses of cortisone, found no decrease in 17-ketosteroid excretion in patients with adrenal cortical carcinoma. Irradiation of the pituitary was insufficient to test the possibility that the metastases were under some degree of anterior pituitary control.

Case 4. I.S. was a young adult male with early onset of puberty, cessation of linear growth at the age of 14, obesity, moon face, abundant body hair, sparse facial hair, small genitalia, no gynecomastia, and high urinary excretion of estrogens, neutral 17-ketosteroids and dehydroisoandrosterone, associated with a large tumor of the left adrenal.

The patient, a student, aged 20 was first seen by us in October 1950, at which time he complained only of excess weight. He had developed normally until 11½ years of age. At that time puberty commenced, with marked growth of pubic, axillary and body hair; but facial hair was, and continued to be, confined to a sparse growth on the chin and upper lip. At age 14, linear growth apparently ceased. From the ages of 16 to 19 he was under the care of Dr. Herman W. Zondek in Jerusalem, who kindly supplied us with the following information covering this period: I.S. showed obesity, especially of the trunk, moon face, red striae, a blood pressure of 90/65 mm. of Hg, and moderately impaired glucose tolerance (see next paragraph); a roentgenogram ("flat plate") showed nothing abnormal in the abdomen and there was no evidence of enlargement of the sella turcica. Dr. Zondek suspected a variant of Cushing's syndrome. In the summer of 1950, I.S. came to the United States to study and consulted us in October 1950 for his obesity. He showed marked growth of pubic, axillary and body hair, but very sparse facial hair, moderate obesity of the trunk, and a plump rosy-cheeked face (Fig. 1c). The testes and penis were smaller than normal for his age. The prostate was normal to palpation. There were no purple or red striae, and no gynecomastia or acne. Nipples and areola appeared normal. His height was 61⅛ inches (152.8 cm.), span 62 inches (155. cm.) and his weight was 175 pounds (79.6 Kg.). The systolic blood pressure varied between 105 and 125 and the diastolic pressure between 60 and 75 mm. of Hg. The rest of the physical examination was not remarkable. Because of the peculiarities of hair growth and his suggestive facies, urinary hormone assays were carried out (Table 1). The striking increase in estrogens and neutral 17-ketosteroids led to further detailed study in the hospital.

Laboratory studies showed normal or negative findings from a blood count, urinalysis, determination of blood urea nitrogen and serum cholesterol, and serologic tests for syphilis. The results of five-hour glucose tolerance tests were as follows:

	Prior to 1949	Oct. 12, 1950	Dec. 21, 1950
Fasting	77 mg./100 ml.	55	73
30 min.	152	116	94
1 hr.	204	110	91
2 hrs.	152	92	109
3 hrs.	113	77	71
4 hrs.	—	35	45
5 hrs.	—	65	66

The intravenous urogram revealed a smooth round mass the size of a large orange in

the left upper quadrant, apparently above and anterior to the left kidney; otherwise the urogram findings were normal. Roentgenograms showed the skull and gastro-intestinal tract to be normal.

On February 10, 1951, Dr. Nicholas Gimble removed a left adrenal tumor through a left subcostal incision (see pathologic report). The large amount of fat in the abdomen made estimation of the size of the right adrenal difficult. Preoperative preparation with cortical extract was apparently not adequate, because eighteen hours after operation there was a rise in temperature and pulse and a fall in blood pressure. The administration of intravenous fluids and large amounts of adrenal cortical extract and cortisone was followed by satisfactory response. Cessation of substitution therapy resulted in another mild crisis, so this therapy was resumed.

Following discharge from the hospital on March 5, 1951, the patient took 25 mg. of cortisone per day by mouth for almost three months and 5 mg. of desoxycorticosterone acetate intraorally each day during the first month. He felt well but his blood pressure was low (80/50) and his appetite decreased. Since stopping substitution therapy on May 26, 1951, he continued to feel well and to have normal energy, despite his continued low blood pressure. During the summer, he was physically active as a counselor in a boys' camp. On October 5, 1951, he weighed 145 pounds (65.9 Kg.)—a loss of 30 pounds (13.6 Kg.) in nine months (Fig. 1 d). His blood pressure was 90/50 mm. of Hg but he was in good physical condition. By March 14, 1952, twelve months after operation, his weight had increased by 14 pounds (6.4 Kg.), but urinary hormone assays continued to be normal.

Pathologic Report. The surgical specimen (85373) consisted of an encapsulated, spherical mass weighing 330 Gm. and measuring 10×10×8 cm. On section, fibrous appearing septa divided pale brown and yellow tissue into irregular lobules. Blood-filled spaces were numerous. A few foci of gritty, cream colored material were also present. Microscopy disclosed masses of loosely packed, large polygonal cells having abundant, eosin-staining cytoplasm. Nuclear pleomorphism was marked. Between the masses of cells were thin-walled vascular channels. A few foci of necrosis and calcification were present. No vessel or capsular invasion was seen. The diagnosis was adenoma of the left adrenal cortex.

Comment. The features of particular interest in this case were the absence of gynecomastia associated with a high urinary excretion of estrogens, neutral 17-ketosteroids and dehydroisoandrosterone, and the development of a syndrome resembling Cushing's disease which partially disappeared prior to removal of the tumor. During his adolescence, he showed a diabetic type of glucose tolerance curve, moon face, red striae, and obesity, but a low-normal blood pressure. The red striae were not present at the age of 20, but a rounded face and bright red cheeks with sparse facial hair were noted. In addition, the glucose tolerance test now showed hypoglycemic values, without associated symptoms. The reason for this post-glucose hypoglycemia is not clear (compare Case 3). At this time, the tumor of the adrenal was visible in roentgenograms, although it had not been visible several years before. This case illustrates, in one patient, the variability of symptoms associated with hyperfunction of the adrenal cortex. It also demonstrates that relatively few and undramatic symptoms may be the only clinical evidence of an actively secreting tumor.

Although the breasts were fat in this young man, there was no evidence of firm mammary tissue by palpation. It is possible that high levels of androgens may have inhibited the effect of the increased estrogens upon the growth of mammary tissue in this patient, as well as in Patient 3. Androgens have been shown to inhibit the growth of mammary

ducts produced in castrated male mice by estrogen (12). That this relationship is a complicated one is indicated by the growth of breast tissue that may occur during administration of testosterone propionate or desoxycorticosterone, and by many other peculiarities of gynecomastia.

DISCUSSION

The literature concerning feminizing adrenal cortical tumors in males (10 cases) was reviewed by Wilkins (13) in 1948, with the addition of 2 new cases. The urinary excretion of estrogens was determined in only 4 of these cases. In 2 instances (Wilkins' #6 and #9), the values for estrogens were many times that for normal men, but the value for androgens by biologic assay was only slightly increased. In one case (Wilkins' #11), there was a moderate increase in urinary estrogens associated with high values for neutral 17-ketosteroid excretion, particularly dehydroisoandrosterone. In another case (Wilkins' #12), a 5-year-old boy showed a questionable increase in estrogen excretion associated with a slight increase in urinary neutral 17-ketosteroid. Both of our cases of "feminization" in males (Cases 1 and 2) showed a marked increase in urinary estrogen excretion without an increase in neutral 17-ketosteroid excretion. In Case 2 the tumor was difficult to classify (see comment on Case 2). Gynecomastia was present in both these men.

Of our other 2 patients, one, a woman (Case 3), showed marked virilization with metabolic disturbances and the other, a man (Case 4), presented primarily the problem of obesity. In both these instances there were high urinary excretion values for estrogens (as determined by assay of the "phenolic fraction"), neutral 17-ketosteroids and dehydroisoandrosterone. The woman (Case 3) had atrophic breasts and the man (Case 4) had no evidence of palpable breast tissue. The possible inhibitory effect of androgens upon the growth of breast tissue induced by estrogens has been discussed in the comments following presentation of these cases.

The clinical and hormonal aspects of virilism have recently been reviewed (14). The urinary excretion of "estrogenic substances" in some patients with the virilizing syndrome has been recognized for many years (15). However, it seems doubtful that the reported "estrogenic effect" in some instances of virilism was due to the true estrogens (estradiol, estrone, or estriol). In at least 1 case, biologic assays have shown a high estrogen titer on the unfractionated extract and a normal titer when the alkaline fraction ("phenolic fraction") was employed (16). It seems possible that dehydroisoandrosterone and possibly other androgens (3) are responsible for the estrogenic effect noted in bioassays of the unfractionated extracts. Demonstration of an estrogenic effect, using the "phenolic fraction," enhances the probability that such effects are due to one or more of the true estrogens.

Histologic diagnosis of adrenal cortical tumors

The distinction between benign and malignant adrenal cortical tumors is, in many instances, quite difficult. Certain of the malignant tumors may readily be identified as such, but assurance of the benign nature of any tumor is often incomplete. Cellular atypicality, pleomorphism, vascularity and mitotic activity may be no greater in carcinoma than in adenoma. The presence of tumor cells in blood vessels is not necessarily an indication of metastasis. The best criteria for the determination of malignancy are the invasion of the tumor through the capsule and the direct growth of the tumor through the walls of blood vessels, in contradistinction to the mere finding of tumor cells floating free in the lumina of vessels. If these criteria are accepted, our Cases 1, 2, and 4 are to be classified as adenomas and Case 3 as carcinoma. However, as 9 of the 12 cases of feminizing adrenal cortical tumors in males mentioned by Wilkins (13) died of metastases, final diagnosis in our 2 living patients must await further observation.

There is no agreement in the literature regarding the relationship of the morphology of these tumors to their hormonal activity. It would appear that the divergent opinions result from the inadequacy of the histochemical methods applied to the study of these lesions.

SUMMARY

1. Four cases are reported of adrenal tumors associated with a high urinary excretion of estrogens. In the 2 patients without increased urinary excretion of neutral 17-ketosteroids, there was gynecomastia. In the other 2 patients there was a marked increase in urinary excretion of neutral 17-ketosteroids and dehydroisoandrosterone. One of these patients (a woman) exhibited virilization, atrophic breasts, hypoglycemia and other metabolic disturbances. The other (a man) had no gynecomastia but showed a number of mild deviations from normal, with obesity as the outstanding feature.

2. Some considerations are discussed regarding: (a) the presence or absence of gynecomastia in these patients, (b) the assay for urinary estrogens in the presence of high levels of neutral 17-ketosteroids, and (c) the histologic diagnosis of adrenal cortical carcinoma.

Acknowledgment

The authors are indebted to Walter Applin and Irmgaard Landolt for technical assistance in performing the hormone assays.

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THE DISAPPEARANCE OF INFUSED AMINO ACIDS FROM PLASMA OF HOSPITALIZED CONTROL AND ADDISONIAN SUBJECTS*

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THERE is considerable evidence that secretions of the adrenal cortex accelerate amino acid catabolism (1), although there is contrary evidence and opinion (2). Cagan, Grey and Jensen demonstrated that when adrenalectomized rats are given amino acids in the form of casein hydrolysate, the elevation in the blood amino-acid nitrogen is greater and more prolonged than that in normal animals (3). Engel showed decreased urea formation in nephrectomized adrenalectomized rats given amino acids in combination with glucose (4), and Parson demonstrated increased conversion of amino acids to urea in Cushing's syndrome (5).

In an attempt to substantiate in human subjects the observations of Cagan, Grey and Jensen (3) and to determine, if possible, whether the abnormality was due to impaired renal function or to defective amino-acid catabolism, the following investigation was performed.

MATERIAL AND METHODS

Cases of Addison's disease (Table 1) were selected as experimental subjects, since these were the available clinical material most comparable to adrenalectomized animals. Two of these (Cases 20 and 26) were recently diagnosed untreated cases, but the others[1] were all known cases being treated with maintenance doses of desoxycorticosterone acetate (DCA). Case 20 was reinvestigated later after treatment with cortisone had been instituted—10 to 15 mg. per day for three months. At no time was any patient in a state of peripheral circulatory failure.

Two control groups were investigated (Table 2). The first group consisted of 13 patients in no apparent stress and without detectable hepatic or renal disease, and the second group consisted of 6 patients receiving cortisone for various reasons. The subjects in this latter group were

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[1] We are indebted to Drs. Louis Soffer and Selig Zorkin of Mt. Sinai Hospital, New York, for their kindness in permitting us to study Cases 21 through 25.

treated with cortisone for periods varying from seven to twelve days. Each was given 100 milligrams of the drug during the 24-hour period preceding the experiment and none received a total dosage less than 1,000 or more than 1,800 milligrams of cortisone. It is true that this group was not an ideal control, since only 1 patient in the Addisonian group was treated with cortisone and 6 with DCA. However, the needs of these subjects made it inadvisable to omit or alter their medication.

TABLE 1. ADD'SON'S DISEASE

Subject No.	Known duration of Addison's disease	Control medication		
		Salt (Gm./dy.)	DCA (mg./dy.)	Cortisone (mg./dy.)
20	New case	0	0	0
21	7 years	3	1.0	0
22	3 years	0	0.5	0
23	11 years	9	1.0	0
24	1 year	3	4.0	0
25	17 years	0	2.5	0
26	New case	0	0	0
27	3 years	2	2	0
20A	¼ year	0	0	10–15

Subjects were confined to bed and were not permitted food for fifteen hours prior to the experiment. Water was given as desired, until one last glass was given 100 minutes before the infusion of casein hydrolysate.

After blood was drawn, 120 milliliters of a 10 per cent casein hydrolysate[2] solution was administered intravenously during a period of twelve minutes. (The casein hydrolysate was found to contain 825 mg. of amino nitrogen per 100 ml.) Blood samples were taken at the following time intervals subsequent to the end of infusion: 5, 15, 30, 55, and 85 minutes; and in several cases in each group at 120- and 240-minute intervals.

Urine analyses for amino nitrogen were performed on the fasting, 90-minute and 240-minute specimens in 13 of the control cases (including 5 of the cases treated with cortisone) and in 7 of the Addisonians.

The fasting urine was obtained just before the infusion was begun. All urine specimens were collected during controlled time intervals. Their volume was measured and the urine saved for analysis. Only females were catheterized routinely; the males were catheterized only when they had difficulty urinating.

Plasma amino-acid nitrogen was measured by the method of Frame, Russell and Wilhelmi (6), and urinary amino-acid nitrogen by the method of Cagan, Goldberg and Loewe (7).

RESULTS

Figure 1 shows the differences in elevation of the plasma amino-acid nitrogen level between the control and the Addisonian groups after infu-

[2] Amigen was obtained through the efforts of Dr. Warren Cox of Mead-Johnson Co.

TABLE 2. COMPARISON OF PLASMA AMINO NITROGEN ELEVATIONS IN THREE GROUPS OF SUBJECTS AFTER CASEIN HYDROLYSATE INFUSION

Subject No.	Age (yrs.)	Sex	Calc'd. plasma vol. (liters)	Total amino nitrogen elevation (mg.) at:	
				5-min. interval	55-min. interval
			Untreated controls		
1	21	M	3.20	99	16
2	35	M	3.55	117	11
3	42	M	3.42	113	21
4	29	M	3.08	99	12
5	31	M	3.35	94	3
6	19	M	3.62	119	4
7	42	F	2.64	74	18
8	16	M	2.95	88	21
9	55	M	3.28	105	20
10	19	M	3.53	106	25
11	32	F	3.32	113	23
12	25	M	3.99	124	28
13	52	F	2.72	114	19
		Mean		*105*	*17*
			Controls receiving cortisone		
14	51	F	3.08	114	9
15	26	M	3.40	82	7
16	54	M	3.20	102	13
17	34	F	3.04	85	24
18	28	F	2.58	100	5
19	26	F	3.13	125	22
		Mean		*101*	*13*
			Subjects with adrenal cortical insufficiency		
20	56	F	2.64	268	42
21	47	F	2.92	129	23
22	24	F	3.36	128	57
23	42	M	3.48	118	35
24	48	F	3.30	126	43
25	45	M	3.01	105	33
26	49	F	3.04	158	49
27	62	F	2.42	174	48
20A	56	F	2.64	124	45
		Mean		*147*	*43*

sions of casein hydrolysate. The average elevation above the fasting level at the 5-minute interval for the untreated control group was 3.2 mg. per cent, with a range of 2.8 to 4.2 mg. per cent. The control group receiving cortisone showed a mean elevation of 3.3 mg., with a range of 2.4 to 4.0. Corresponding values for the Addisonian group were 5.1 mg. per cent, with a range of 3.4 to 10.2 mg. per cent.

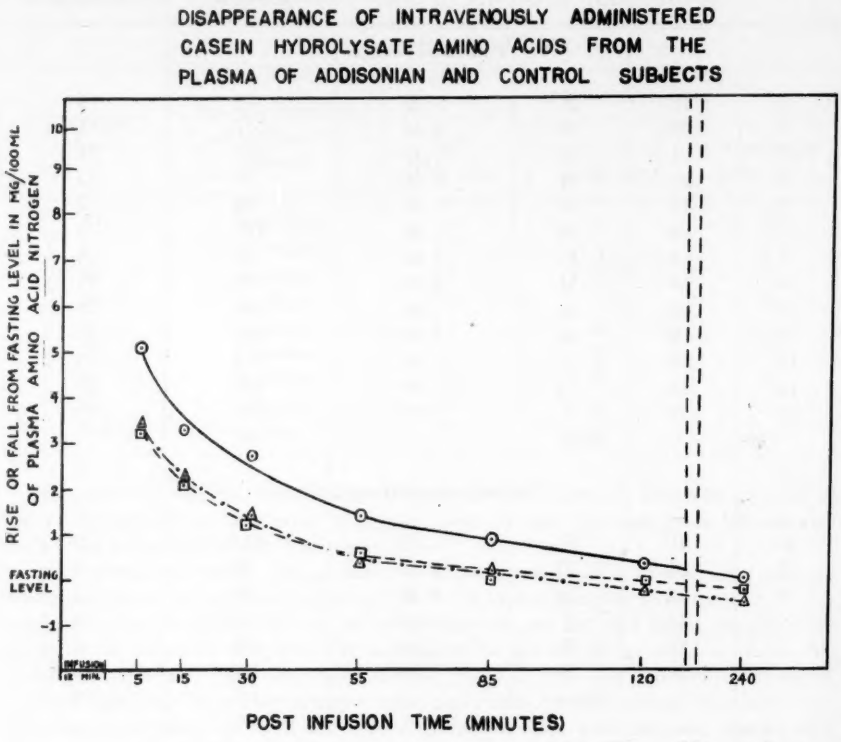

FIGURE 1

The average subject in the experimental group was smaller than those in the control groups; therefore, elevations of the plasma amino nitrogen level were corrected for each patient's plasma volume. Table 2 shows the total elevations in plasma amino-nitrogen levels after this correction.

At the 5-minute interval, in the untreated controls, the value for the

total plasma amino-nitrogen elevation above the fasting level averaged 105 mg. The experimental group showed an average increase of 147 mg. The standard deviations of the groups were 3.9 and 15.7, respectively. At the 55-minute interval, the mean for the untreated control group elevation was 17 mg., whereas the same value for the experimental group was 42 mg. The respective standard deviations were 2.3 and 3.2. Values for the control group treated with cortisone showed little significant variation from those for the untreated controls.

These data, analyzed in accordance with Fisher's technique (8), showed significance, with a p value of less than 0.01 for both the 5-minute

TABLE 3. INCREASE ABOVE FASTING LEVEL OF URINARY EXCRETION OF AMINO NITROGEN FOR THE 240-MINUTE PERIOD AFTER INFUSION, IN THREE GROUPS OF SUBJECTS

Controls		Controls receiving cortisone		Addisonians	
Subject No.	Excess amino nitrogen (mg.)	Subject No.	Excess amino nitrogen (mg.)	Subject No.	Excess amino nitrogen (mg.)
6	8	14	5	20	7
7	20	16	6	21	8
8	19	17	6	22	20
9	10	18	0	23	0
10	6	19	0	24	6
11	7			25	8
12	4			26	4
13	13			20A	0
Mean	*10.9*	*Mean*	*3.4*	*Mean*	*6.6*

and the 55-minute plasma amino-acid elevation. However, when only the DCA-treated Addisonians were compared with the untreated controls, the p value at five minutes was 0.1 but was still significant at 55 minutes (less than 0.01).

A second abnormality is seen in patients with Addison's disease. Their plasma amino-acid elevation persisted until the 120-minute interval, when the level approached the fasting mark (Fig. 1). However, in the control groups, the reversion to the fasting level occurred at the 85-minute interval.

In Table 3 are compared the amounts of urinary amino nitrogen excreted above fasting levels, in the control and experimental groups after

infusion of the casein hydrolysate. The untreated controls excreted an average of 4.3 mg. more amino nitrogen than did the Addisonian group, but the group receiving cortisone excreted still less of the infused amino acids than did the Addisonian group.

DISCUSSION

In the Addisonian group, the greater and more prolonged elevation above fasting of plasma amino-acid nitrogen may be an expression of the decreased rate of catabolism. The absence of kidneys in Engel's animals may partly explain his inability to obtain, consistently, a more rapid return to normal of blood amino acids in his control nephrectomized rats than in his nephrectomized-adrenalectomized rats (4). The kidney is known to be a site for the oxidative deamination of amino acids and this oxidation has been shown to be accelerated by adrenal cortical secretions (1, 3).

On the other hand, the decreased tolerance for amino acids may be a manifestation of an increased tubular reabsorption of amino acids in the Addisonian group. Some decrease in excretion of amino acids was noted in these patients, but a still greater diminution was noted in the control group receiving cortisone, and this group showed no abnormal or prolonged plasma elevation. Therefore, it is possible that the decreased excretion of amino acids in the Addisonian group represents the superimposed effect of medication. This thought is substantiated by the data in Case 20, in which the post-infusion increase in amino-acid excretion was no longer observed after treatment with cortisone. Whether or not the cortical steroids cause increased tubular reabsorption of amino acids cannot be stated with certainty at this time, but there is evidence that the adrenal cortical steroids may act on the kidney in this manner (9).

It is felt that the greater fall (in milligrams) of the amino acids in the Addisonian patients during the period from 5 minutes to 55 minutes following infusion (Table 2) represents not greater catabolism, but greater capillary permeability. Since the cortical steroids are known to decrease capillary permeability (10), it is probable that the more rapid disappearance of amino acids in the Addisonian group is a manifestation of the lack of cortical steroid.

Although indicative of a defect in the Addisonian's ability to handle amino acids, these data do not clearly relate the defect to impaired amino-acid catabolism. Nevertheless, it is possible that this deviation may be due to such a defect, resulting from an insufficient cortical steroid supply.

SUMMARY

Twenty-seven hospitalized subjects were given 120 ml. of a 10 per cent casein hydrolysate solution intravenously in twelve minutes. Plasma and

urine were examined at regular intervals for amino acids.

There were 8 established cases of Addison's disease, of which 6 were treated with desoxycorticosterone acetate, and 2 were untreated; one of the latter was reinvestigated after treatment was instituted. There were 6 control subjects with varied diseases treated with cortisone, and 13 other control subjects without renal or hepatic disease and under no physical stress.

The Addisonian group showed a higher and more prolonged rise in the level of plasma amino-acid nitrogen than did either of the control series.

This abnormal plasma amino-acid elevation in the Addisonian group may be accounted for by defective amino-acid catabolism.

It is possible that an effect of adrenal cortical steroids on renal function was manifested by the treated Addisonian and the treated control subjects, who excreted less of the infused amino-acid nitrogen than did the untreated controls.

Acknowledgment

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THE EFFECT OF ORALLY ADMINISTERED ASCORBIC ACID AND/OR *l*-CYSTINE ON URINARY STEROID EXCRETION*

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THERE is considerable evidence that ascorbic acid and sulfhydryl-bearing compounds influence adrenal cortical function. The relationship between ascorbic acid and adrenal activity is well known and need not be reviewed here, except for a few pertinent data. Massive dosage of ascorbic acid prevents adrenal hypertrophy of animals exposed to the stress of low temperatures, and increases their survival rate (1). The rise of urinary 11-oxycorticoid excretion is less when ACTH is given in combination with ascorbic acid than when ACTH is used alone (2). Even in scorbutic patients the administration of ascorbic acid produces a temporary decrease in the already low 11-oxycorticoid excretion (3). Depletion of adrenal ascorbic acid under rigidly controlled circumstances serves for the bioassay of ACTH.

The adrenal cortex is rich in sulfur-bearing, nonprotein compounds including glutathione, dimethyl sulfone, taurine, bis-β-hydroxyethyl sulfoxide, potassium ethyl sulfate and other incompletely identified substances. A high rate of organic sulfur (4) and cystine-S^{35} (5) pickup and storage has been demonstrated by various techniques. Adrenal sulfhydryl concentration has been shown to vary under the stimulus of ACTH (4a) and blood sulfhydryl levels are altered in Addison's disease (6) and in sodium-deficient rats (7); some workers have reported a decrease in sulfhydryl levels on administration of ACTH in man (4b,8, 9). The need for investigating this mechanism of a relationship between organic sulfur, ascorbic acid, and adrenal cortical function has become increasingly evident. We therefore studied the effect of oral ascorbic acid and/or *l*-cystine administration on urinary steroid excretion in man.

MATERIALS AND METHODS

Sixty-eight patients whose urinary steroid excretion had been studied in relation to another problem (chronic pulmonary tuberculosis) were used.

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Although these subjects were obviously not to be classified as normal, their clinical status and level of adrenal function were well known, enabling us to evaluate their response to therapy. The entire group of 49 males and 19 females had been maintained in the same hospital unit, with the same diet kitchen and nursing staff, for a prolonged period of time. The age and sex distribution was as follows: under 20 years of age, 3 females; 21 to 30 years of age, 8 females; 31 to 40 years of age, 4 females and 7 males; 41 to 50 years of age, 2 females and 11 males; 51 to 60 years of age, 1 female and 20 males; and 61 to 70 years of age, 1 female and 11 males. The extent and activity of tuberculosis were classified according to the criteria of the American Tuberculosis Association. There was 1 patient in group 2A, 6 in group 2B, one in group 3A, 9 in group 3B and 43 in group 3C.

Seven patients had a sedimentation rate (Westergren) of less than 10 mm./hr.; 9, a rate of 11 to 25 mm./hr.; 16, a rate of 26 to 50 mm./hr.; 16, a rate of 51 to 75 mm./hr.; 9, a rate of 76 to 100 mm./hr.; and 11, a rate greater than 100 mm./hr.

Blood pressure, body weight, sedimentation rate, eosinophil count, cardiothoracic ratio (10), urinary 17-ketosteroids (11) and neutral reducing corticoids (12) were determined at two-week intervals. The relationship of clinical findings to urinary steroid excretion has been published in detail elsewhere (13).

The urinary 17-ketosteroid values were plotted according to age and sex and compared to normal values obtained by the same technique (14). The values for females were within the normal range; only 4 male patients in the 59–69 age group showed low values, and of these low values, only 2 were substantially depressed. The neutral reducing corticoids were plotted in the same manner. Again, the individual values were within the normal range. It may be concluded that these parameters, when corrected for age and sex, do not indicate marked adrenal insufficiency in this particular group of patients. However, a definite correlation between urinary steroid excretion and tuberculous activity as measured by sedimentation rate was demonstrated. The data in Table 1 show that there is a significant decrease in the average excretion of 17-ketosteroids and neutral reducing corticoids in patients with elevated sedimentation rates. Evidently this factor must be taken into consideration when the subsequent results are evaluated.

The presence or absence of weight loss, the occurrence of hypotension or diminished cardiothoracic ratio, or changes in the total eosinophil count of these patients showed no significant correlation with urinary steroid excretion.

Two schedules of treatment were used. In one, ascorbic acid (1 gram

TABLE 1. CORRELATION BETWEEN URINARY STEROID EXCRETION AND BLOOD SEDIMENTATION RATE

Sed. rate in mm./hr.	Number of patients	Average excretion in mg./day	Significance (t-value)
	Ketosteroids		
0–30	14	18.6±7.0	2.1
over 50	32	14.5±5.9	
	Neutral reducing corticoids		
0–30	19	3.5±1.05	2.0
over 50	33	2.9±0.99	

orally per day divided into three doses) was given for eight weeks, and during the last four weeks *l*-cystine (2 grams per day divided into three doses) was given simultaneously. In the other schedule the situation was reversed: a daily dose of 2 grams of *l*-cystine was given for eight weeks and during the last four weeks 1 gram of ascorbic acid daily was added. The 68 patients were divided into small subgroups of from 4 to 6, and new groups were started on alternate schedules every fourth day. In this way it was hoped that temporal or consistent technical errors would be put on a random basis as much as possible. Physical measurements, laboratory studies and urine collections were carried out before the institution of therapy, and every two weeks thereafter. The laboratory workers were not aware of the agents used in this investigation.

RESULTS

The changes in urinary steroid excretion under treatment are shown in Table 2.

Effect of l-cystine therapy. There are two groups of patients who received cystine for four weeks. One group had no previous treatment, whereas the other had been pretreated for four weeks with ascorbic acid and was maintained on this therapy throughout *l*-cystine treatment. Both groups showed a consistent drop in urinary 17-ketosteroid and neutral reducing corticoid excretion. The trends are shown graphically in Figure 1.

Further statistical study was required to establish the significance of this observation. Patients were divided into those showing a drop from the initial level after four weeks of *l*-cystine administration versus those showing no drop. This distribution was then evaluated by the statistical sign test, which is based on compound probability alone. Forty-four patients

TABLE 2. CHANGES IN URINARY STEROIDS UNDER TREATMENT

Duration of previous treatment, in weeks→	0	2	4	4	8
L-cystine, 2 Gm./day		■	■	■	
Ascorbic acid, 1 Gm./day				■	■
17-Ketosteroids, mg./day Males	15.3±5.7 (23)*	15.4±4.5 (23)	15.8±6.1 (24)	12.1±5.0 (22)	12.3±4.0 (22)
Neutral reducing corticoids, mg./day Males	3.2±1.0 (20)	2.4±0.6 (19)	3.4±1.1 (19)	2.8±1.2 (19)	2.8±1.1 (16)
17-Ketosteroids, mg./day Females	14.2±5.1 (7)	15.2±3.8 (7)	17.8±6.4 (7)	15.6±6.6 (7)	11.4±4.8 (7)
Neutral reducing corticoids, mg./day Females	2.7±0.9 (12)	2.2±0.5 (12)	2.4±0.8 (12)	2.5±0.9 (11)	2.3±0.6 (11)
L-cystine, 2 Gm./day				■	■
Ascorbic acid, 1 Gm./day		■	■		
17-Ketosteroids, mg./day Males	19.4±6.7 (19)	17.5±6.5 (19)	17.0±6.3 (19)	15.8±6.0 (18)	18.0±8.4 (17)
Neutral reducing corticoids, mg./day Males	3.5±1.2 (27)	3.1±1.3 (27)	3.0±0.9 (24)	3.7±1.5 (20)	4.6±1.6 (21)
17-Ketosteroids, mg./day Females	12.4±3.9 (12)	9.8±4.1 (12)	11.3±5.0 (12)	9.0±2.1 (11)	10.3±5.2 (10)
Neutral reducing corticoids, mg./day Females	3.3±1.6 (7)	2.5±1.1 (7)	2.2±0.8 (7)	2.1±0.2 (7)	2.3±1.4 (7)

* Figures in parentheses indicate number of patients.

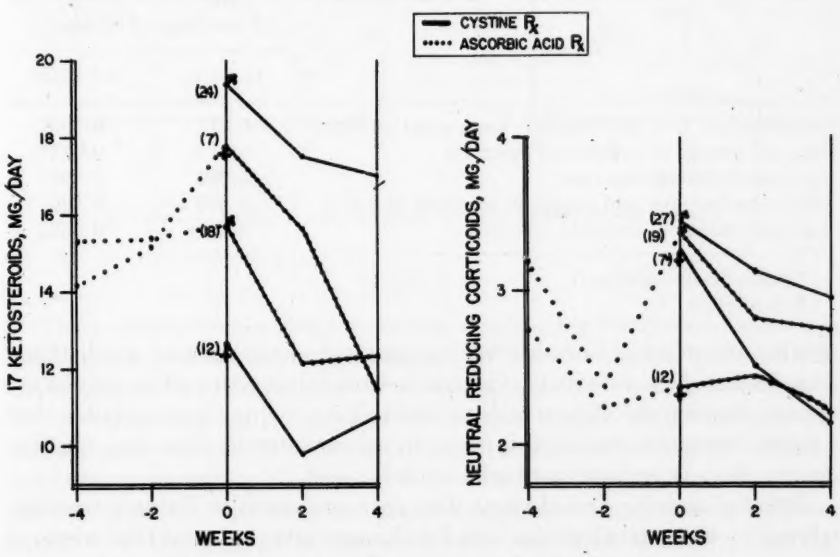

FIG. 1. Effect of *l*-cystine on urinary steroid excretion.

showed a drop in 17-ketosteroid excretion; 16 did not. This distribution is significant at the 1 per cent level. Thirty-nine patients showed a drop of neutral reducing corticoid excretion; 21 did not. This is significant at the 5 per cent level. Then a test was made of the parallelism between the changes in ketosteroid and corticoid excretion: in 43 instances the values changed in the same direction and in 17 they did not. This relationship is significant at the 1 per cent level.

There are, however, distinct possibilities that other variables such as age and sex distribution, or tuberculous activity, might affect these results. A more detailed statistical analysis under the supervision of Dr. Donald Mainland was employed to test these possibilities. Linear regression analysis applied separately to males and females, with subsequent elimination of possible age effects, was carried out separately for both urinary metabolites. The correlation coefficients used to test the significance of the regression relationships are given in Table 3. The only significant finding is that of a correlation between sedimentation rate and change in ketosteroid excretion in females; this value is significantly different from the value for males (tested by interclass z transformation). This indicates that, on the average, the greater the sedimentation rate (*i.e.*, the heavier the adrenal stress), the less *additional* reduction of ketosteroid excretion will be produced by *l*-cystine. These analyses seem to prove that, in these

TABLE 3

Variates	Correlation coefficient	
	41 males	18 females
Sedimentation rate, and change in ketosteroid excretion	−0.077	0.563*
Age, and change in ketosteroid excretion	0.215	0.463†
Age, and sedimentation rate	−0.013	0.296
Sedimentation rate, and change in corticoid excretion	0.096	0.022
Age, and change in corticoid excretion	0.0099	0.0632

* Statistically significant.
† Borderline.

groups, the effect of *l*-cystine was independent of the age and sex distribution, and that the tuberculous process lessened the cystine effect only in the group showing the highest sedimentation rate. It may be concluded that cystine treatment causes a decrease in urinary steroid excretion, whether or not there is pretreatment with ascorbic acid.

Effect of ascorbic acid therapy. The data presented in Table 2 were analyzed by the statistical sign test for changes after two and four weeks of treatment. No significant changes in urinary ketosteroid excretion were

found at either time. After two weeks of ascorbic acid administration 25 patients showed a drop in neutral reducing corticoid excretion and 9 did not; this is significant at the 5 per cent level. After four weeks of treatment this trend was reversed (Fig. 2). There was one other finding of interest: in 22 of 25 patients whose neutral reducing corticoid excretion had been depressed by four weeks of *l*-cystine treatment, ascorbic acid caused a rise in excretion which was significant at the 1 per cent level. The effects on ketosteroid excretion were not significant.

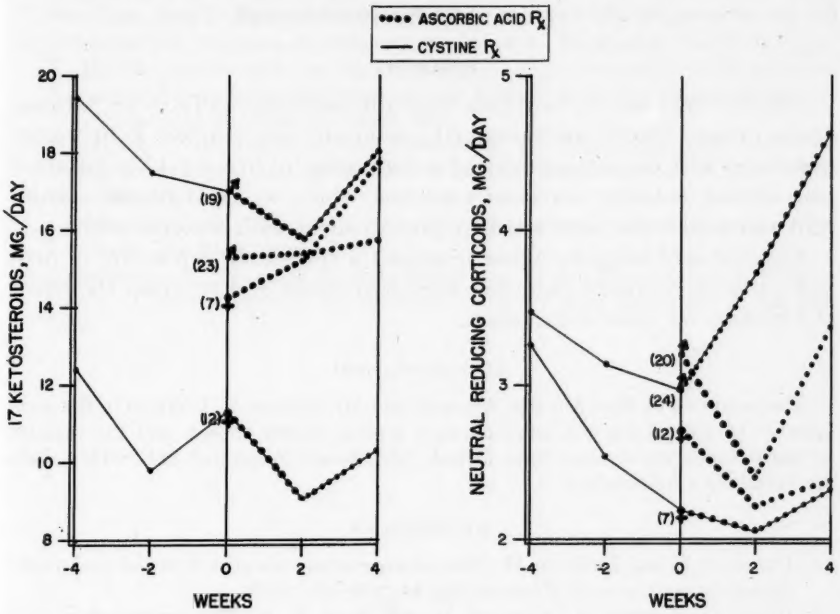

FIG. 2. Effect of ascorbic acid on urinary steroid excretion.

DISCUSSION

These studies demonstrate a definite relationship between *l*-cystine administration and urinary steroid excretion. The mechanism by which this depression occurs is unknown. It is possible that 1) *l*-cystine interferes in the reaction between ACTH and the adrenal cortex, 2) the steroids are utilized more efficiently in the periphery, thereby decreasing the demand for cortical secretion, 3) the metabolism of the elaborated hormones is altered in such a way that the examined urinary metabolites are diminished, or 4) the renal excretory mechanism is altered. We have observed acute changes in the sulfhydryl level of adrenal tissue and peripheral blood

upon increasing adrenal function (by administration of ACTH); accordingly it is unlikely that changes in peripheral metabolism of adrenal steroids or of urinary excretion of their metabolites are responsible for the phenomena observed.

The transitory depression of corticoid excretion upon treatment with ascorbic acid confirms the observations of others already cited. The experiments on stressed animals suggest the possibility that ascorbic acid exercises either a peripheral synergism with cortical hormones or some form of substitutive effect. We cannot offer any explanation at the present time for the reversal of the cystine effect by ascorbic acid.

SUMMARY

Ascorbic acid and/or *l*-cystine were administered orally to 68 patients whose urinary steroid excretion had previously been studied. Four weeks' treatment with *l*-cystine produced a depression of urinary 17-ketosteroid and neutral reducing corticoid excretion which was statistically significant and which was unaffected by pretreatment with ascorbic acid.

Ascorbic acid administration produced a transitory depression of neutral reducing corticoid excretion after two weeks and reversed the effect of *l*-cystine on steroid excretion.

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The assistance of Miss Annabel Schuman and Mr. Edward L. Richman in the management of the nursing and administrative aspects of this project, and the valuable technical assistance of Miss Kate Pollack, Mr. Robert Scism and Mr. Sidney Stern are gratefully acknowledged.

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THE DETERMINATION OF PROTEIN-BOUND IODINE BY DRY ASHING

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THE relation of thyroid function to circulating iodine in the human appears to be well established (1–6) but the technical difficulties inherent in the determination of amounts of iodine of the order of 0.02 gamma have discouraged the universal adoption of existing methods (7, 8) as routine diagnostic aids. The methods most commonly used are based on Chaney's (9) procedure and involve acid digestion of the washed serum protein, reduction, distillation and absorption of the evolved iodine, and finally its colorimetric determination by its catalytic effect on the reduction of ceric sulfate by arsenious acid.

To eliminate the constant attention required in the acid digestion procedures, Barker has recently introduced (10) an alkaline ashing method with subsequent acid leaching of the iodine from the ash. Considerable simplification is thereby attained, since no attention is required during the two to three-hour incineration period. However, in order to rule out the possible effects of impurities on the colorimetric determination, a further complication was introduced which prevents the determination of more than 3 specimens, in duplicate, simultaneously. Our own experience seems to indicate that nothing in the incinerated ash influences the colorimetric determination except possibly the excessive amount of salt formed during the leaching. If a standard curve is prepared from iodide solutions containing similar amounts of sodium chloride, results comparable to those after acid digestion are consistently obtained.

The procedure outlined here has been in use in this laboratory for more than a year, and enables one analyst to determine the protein-bound iodine (PBI) content of about 20 specimens in duplicate per day.

METHOD

Reagents

Water: redistilled from sodium hydroxide; used in all subsequent work.
Zinc sulfate: 10 per cent, of the heptahydrate.
Sodium hydroxide: 0.5 N.
Sodium carbonate: 4.0 N.

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Sulfuric acid: 3.5 N; 98 cc. of the concentrated acid (Sp. Gr. 1.84) per liter.

Hydrochloric acid: 2.0 N; 175 cc. of the concentrated acid (Sp. Gr. 1.18) per liter.

Arsenious acid: Dissolve 3.8 Gm. of arsenic trioxide in 50 cc. of N sodium hydroxide, add 50 cc. of water, then sufficient 3.5 N sulfuric acid to make 500 cc.

Ceric sulfate: Dissolve about 39 Gm. of ceric ammonium sulfate in a liter of 3.5 N sulfuric acid. Exactly 0.5 cc. of this solution mixed with 5.5 cc. of water in a colorimeter tube should give a reading of about 500 in the Klett-Summerson colorimeter (water "zero," blue #42 light filter). If the color is too weak, add more solid ceric ammonium sulfate; if too strong, dilute with 3.5 N sulfuric acid to the desired color intensity.[1]

Apparatus[2]

Muffle furnace: Hoskins electric, type FD204.

Temperature controller: Temcometer equipped with Chromel-alumel thermocouple and indicating dial.

Photoelectric colorimeter: Klett-Summerson.

Water bath: Electric, constant temperature.

Hot air oven: Inside dimensions about 11 inches deep, 19 inches wide and 11 inches high.

Procedure

Into a lipped Pyrex test tube (25×100 mm.) are pipetted 7 cc. of water and, with mixing, 1 cc. each of serum, 10 per cent zinc sulfate and 0.5 N sodium hydroxide. After standing about five minutes, the protein is compacted in the centrifuge, the supernatant liquid discarded and the protein then washed twice in the centrifuge with 10-cc. portions of water. The washed protein is suspended in 1 cc. of 4 N sodium carbonate by means of a fine glass rod, which is removed after rinsing with 0.5–1.0 cc. of water; the carbonate-protein mixture is dried overnight in a hot air oven[3] at 90° C. The test tube containing the dried powder, supported in a suitable rack[4] is placed in a muffle furnace. Incineration is carried on for 2.5 hrs. at 600° C. (± 20°).

To the cooled residue in the test tube are cautiously added exactly 2.5 cc. of 2 N hydrochloric acid and the contents swirled occasionally during the course of ten minutes. At this time all the white residue is usually dissolved and the odor of hydrogen sulfide dissipated. Exactly 10 cc. of water are now added, thoroughly mixed with the contents of the tube, and then centrifuged briefly to pack down any insoluble residue.[5] The supernatant liquid is poured off into a clean dry test tube.

[1] Ceric ammonium sulfate varies in moisture content; the amount to be used for any one lot is best determined in this way.

[2] Obtainable from Arthur H. Thomas Co., Philadelphia, Pa.

[3] To minimize the possibility of iodine contamination all glassware is kept in this oven until shortly before use.

[4] A rack constructed of monel metal or other high nickel steel is satisfactory; stainless steel has a tendency to flake on repeated exposure to high temperatures.

[5] There usually remains undissolved a small but variable amount of carbon, as well as some silica.

Two 5-cc. portions are pipetted into colorimeter tubes, 0.5 cc. of the arsenious acid solution added and the tubes placed in a constant temperature water bath at 37° C. Exactly 0.5 cc. of the ceric sulfate solution is now added, and thereafter at predetermined intervals. Since the decolorization of ceric sulfate is continuous, a precise time schedule must be adopted and strictly adhered to in all the work. In this laboratory we prefer to add the ceric sulfate at one-minute intervals and make the colorimetric readings exactly fifteen minutes thereafter. This permits of unhurried readings and the determination of 7 samples in duplicate. A Klett-Summerson colorimeter with a blue (#42) filter and a water "zero" is used. The iodine content is read from a previously prepared standard curve and the result multiplied by 250 to give the iodine content in gamma per 100 cc. of serum.

Preparation of standard curve

An 8.48-Gm. portion of anhydrous sodium carbonate in a 250-cc. Pyrex beaker is heated in the muffle at 600° C. for two and a half hours. To the cooled powder, 100 cc. of 2 N hydrochloric acid are cautiously added and the volume finally brought to 500 cc. with water.

A stock solution of 130.8 mg. of potassium iodide per liter of water is prepared. From this solution (equivalent to 100 gamma of iodine/cc.), and a chloride solution made as previously described, a series of iodine dilutions from zero to 0.1 gamma per 5 cc. are prepared. To duplicate 5-cc. portions of these diluted iodine standards are added 0.5-cc. portions of arsenious acid; the amount of ceric sulfate reduction in fifteen minutes is determined exactly as outlined in the procedure.

The colorimeter readings, plotted on coordinate paper against the respective iodine concentrations, produce a curve such as shown in Figure 1. Since the standard iodine solutions contain an amount of chloride (obtained in the same way) comparable to that of the serum samples, the values obtained reflect this salt, as well as the iodine contaminating all the reagents, except that which may not be washed out of the zinc hydroxide; blanks should, nevertheless, be run periodically and particularly when any reagent is replenished.

EXPERIMENTAL

To ascertain whether iodine is lost during the incineration, known amounts of iodide were added to amounts of washed zinc hydroxide normally present in the serum precipitate. After drying at 90° C. overnight, these samples were incinerated for two and a half hours at 600° C. and the iodine determined as just described. Table 1 indicates that substantially none of the added iodine is lost by the heat treatment. The same table in-

dicates adequate recovery of known amounts of iodine added to washed serum protein.

TABLE 1. EFFECT OF INCINERATION ON PBI

Sample number	Iodine (gamma per 100 cc.)		
	In sample	Added	Found
1	0.0	2.0	2.25
2	0.0	2.0	2.25
3	0.0	4.0	4.25
4	0.0	4.0	4.25
5	0.0	6.0	6.10
6	0.0	6.0	5.9
7	0.0	8.0	7.0
8	0.0	8.0	8.0
9	0.0	10.8	10.0
10	1.1	4.0	5.0
11	5.3	4.0	9.0
12	5.5	2.0	7.5
13	5.5	4.0	9.0
14	5.5	6.0	11.0

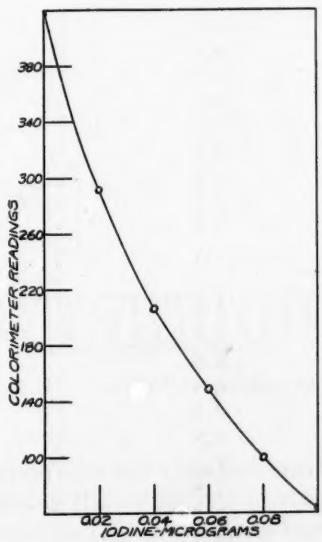

FIG. 1. Relation between Klett-Summerson colorimeter readings and iodine content (15-min. incubation at 37° C.).

The protein-bound iodine content was determined on a group of sera by both the present method and the acid digestion method of Barker (8). The results listed in Table 2 indicate practical agreement between the two methods. The use of sulfuric acid, either alone or in combination with hydrochloric acid, as a leaching reagent for the ash as directed by Barker(10) invariably led to very low results, frequently of the order of 50 per cent of the true values. The reason for this is not clear but it was obvious that sulfuric acid could not be used as part of the leaching solution.

TABLE 2. SERUM PROTEIN-BOUND IODINE CONCENTRATION: COMPARATIVE VALUES

Sample number	PBI (gamma per 100 cc.)	
	Present method	Barker's method*
1	1.3	0.6
2	3.3	4.1
3	3.8	4.7
4	3.9	3.6
5	4.0	3.6
6	4.0	3.8
7	4.1	3.9
8	4.1	4.6
9	4.3	4.7
10	4.3	5.2
11	5.5	5.0
12	4.7	4.4
13	4.8	4.1
14	5.0	4.4
15	5.0	4.9
16	5.0	4.7
17	5.3	5.8
18	5.3	6.6
19	5.5	5.0
20	5.9	6.6
21	6.0	6.1
22	6.0	7.1
23	6.4	5.2
24	6.5	5.8
25	6.8	6.3
26	7.0	5.3
27	7.8	8.5
28	9.7	10.7
29	14.3	15.1
30	15.7	14.7

* Values in this column have been increased by 10 per cent (8).

DISCUSSION

This method for the determination of PBI has been in use for over a year and continued experience indicates that duplicates within 0.5 gamma per 100 cc. may be consistently obtained over the range 1.0 to 16.5 gamma per 100 cc. of serum.

It is worthy of note that unusually high (10) values were not observed as a result of the simplified colorimetric procedure and there would appear to be no necessity for an upward revision of the usual normal values. Indeed an analysis of Figure 2, in which over 1,000 consecutive determinations are listed, would seem to indicate that the normal range for protein-bound iodine, in conformity with more recent work (11, 12), should be between 3.5 and 7.0 micrograms per 100 cc.

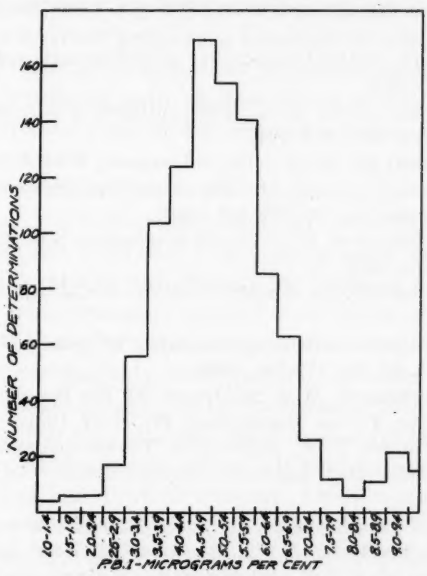

FIG. 2. Range of PBI level in over 1000 consecutive samples of human serum.

Because mercuric iodide does not volatilize, methods employing distillation as a means of isolating the iodine lead to very low results in patients receiving mercurial diuretics (13). Such cases have no effect on the PBI determined by the present method, as shown by analysis of the sera of patients under this treatment, as well as by the addition of mercurials directly to the serum protein before ashing.

SUMMARY AND CONCLUSIONS

A simplified procedure for the determination of protein-bound iodine of serum is presented. It involves dry ashing, and enables the estimation in duplicate of about 20 specimens per day. Normal values for PBI by this method appear to approximate 3.5 to 7.0 gamma per 100 cc. Mercurial therapy does not lead to artefacts.

The validity of the method is indicated by adequate recoveries of added iodine, as well as by similar results obtained on the same sera by both an acid digestion method and the one here described.

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EPILATION BY ELECTROCOAGULATION: IMPROVEMENTS IN TECHNIC

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IN a previous report (1), based on fifteen years of experience in the treatment of hirsutism, reasons were presented for the superiority of electrocoagulation over electrolysis. In the subsequent seven years, during which 300 more patients have been treated, the undamped diathermy machine has been replaced by the spark-gap machine, because the latter has been found to be more proficient. Interrupted rather than continuous application of the current permits more permanent destruction of the hair follicle and a reduction of pain. A description of this newer method of treatment, including a simple device for inserting the needle to the correct depth, is here presented.

A survey of the office records shows that an increasing number of women are seeking relief from hirsutism. No attempt has been made to determine whether the condition is becoming more prevalent (although some possible explanations are offered), or whether the increase is due merely to greater fastidiousness, coupled with better economic conditions than in the previous 15-year period. Ages in this series ranged from 11 to 70 years. Only female patients were treated. One man who requested removal of the beard and another, of the moustache, and a few women who desired epilation of the arm pits and pubic region (either models or ballet dancers) were refused treatment. It was felt that nothing was to be gained by removing normal hair by this method. The process is a tedious one, even when the growth is abnormal and distributed less evenly than is normal hair; moreover, regrowth occurs in an estimated 20 per cent of cases. It is interesting to note that no valid reasons for their requests were given by the men.

Before treatment was instituted, a careful history was obtained in order to discover if any endocrinopathies existed. Many patients were referred by endocrinologists after careful studies with negative results. It is imperative that patients showing virilizing syndromes be thoroughly studied.

As Calloway and associates (2) have pointed out, the virilizing syndrome

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may be accompanied by an unimportant amount of hirsutism, and the converse may also be true.

TECHNIC

The machine now used by the author consists of a special epilation apparatus, with a rigid, noninsulated steel needle about ½ inch long. It is equipped with a foot control, permitting the application or release of the current at will, so that the current can be reduced at frequent intervals. A blunt or ballpoint needle is undesirable, because it cannot be inserted with ease into the hair follicle. The author is in agreement with the opinion that multiple needles are of no advantage (3). Lampblack or other coloring matter may be used to make blond hair more easily seen, but a slight pull with tweezers will usually be sufficient to indicate the location of the follicle.

As Ellis (4) has pointed out, it is not always possible for even an experienced operator to ascertain whether he has inserted the needle too far or not far enough. The author has found that the optimum depth to which it should enter the skin is almost invariably ⅛ of an inch. Until recently he marked this distance on the needle by coating it as far as ⅛ of an inch from its point, with red nail polish. The needle stopped when the rim of polish touched the skin, and the color was an additional aid to the eye. The disadvantage of the polish lies in its brittleness, a quality which causes it to chip readily. After the needle thus marked has been used for a time, the polish must be replaced with a new coat. This older method of marking the needle has been discarded for a combination colorless liquid-cement glue and polyethylene tubing. A thin coat of liquid-cement glue is applied, as with the old method, to within ⅛ of an inch of the point of the needle. Then a sleeve of the tubing (one that will pass through an 18-gauge needle, and previously cut so that it also ends precisely ⅛ of an inch from the point of the needle) is slipped onto the needle and into position. The diameter of the tubing is large enough to permit the end of the needle to be inserted and small enough to fit snugly. The glue holds the tubing in place. The resulting coat of this resilient substance is light, and durable enough to withstand an indefinite number of manipulations for successive treatments of different patients; the smallest length that can be purchased will last for several years. The tubing also makes the needle more rigid.

With the needle in position, the foot switch is pressed for a fraction of a second to three seconds, and this is repeated from two to three times. The length of time required for leaving the needle in position depends on the type of hair being removed and the sensitivity of the patient. On the upper lip, especially in older women whose skin has lost its fat and elasticity, the current should be low. Little or no scarring will result in any area if care is exercised to prevent the needle from becoming too hot or from being inserted superficially.

From 40 to 50 hairs can be removed in five minutes, and the first treatment should not be longer than this. Thereafter the period will vary according to the patient's tolerance. The most important requirement for keeping pain and unpleasant after-effects at a minimum is that the space between the follicles destroyed during a given treatment be not less than ⅛ of an inch. The hairs remaining in these spaces can be removed on the patient's return visit. Treatments may be scheduled at daily intervals and continued until the area is finally epilated. The rate of 40 or 50 hairs in

five minutes compares more than favorably with rates of removal by other methods. Ellis (4) reported that he was able to remove 120 hairs in thirty minutes with a high frequency vacuum tube machine and that the rate of removal by electrolysis is about 40 hairs in thirty minutes. Cipollaro (3) states that "it should not take more than one or two seconds to destroy a hair follicle when the proper machine and the proper current are used."

Reactions, if they occur, are minor and consist of an erythema which disappears rapidly, and occasionally papules and pustules. Bathing with alcohol immediately after the treatment, followed by drying and the application of a skin-colored lotion containing 0.12 per cent mercury bichloride and 1 per cent phenol, will usually prevent these after-effects. If pustules form, aureomycin, terramycin, or one of the other antibiotic ointments should be used to prevent infection. Penicillin ointment is not used becasue of its sensitizing reactions. Epilation has not been performed in members of the Negro race because of possible depigmentation.

Two precautions should be taken. The first is to make sure that the physician's head-light does not rest on his nose, because the irritation resulting from the heat and weight of the light used continuously in this position may lead to changes in his skin, such as telangiectasia and possibly eventual malignancy. Illumination will be sufficiently good to permit insertion of the needle into the follicle, if the light (suspended from a band encircling the forehead) is at least 4 inches from the physician's face. The second precaution is to use only a machine designed for epilation. Cipollaro (3) emphasizes the importance of this factor by stating that "the most important thing . . . is to have equipment which is manufactured for this special purpose. Many dermatologists make the mistake of thinking that any equipment for the use of high frequency current is suitable . . . . It is not, because with some equipment the current cannot be cut down enough to destroy the follicle without also destroying a great deal of surrounding tissue."

Disappointment and lack of confidence in the physican will be forestalled if the patient is told that a few hairs may regrow. In that event, the patient should return for retreatment as soon as possible, and before full growth is attained, since partly regenerated hair follicles are destroyed more readily than are fully grown follicles (4). The high rate of regrowth should not be construed as an indication of the ineffectiveness of the method or of epilation in general.

DISCUSSION

Time spent in removing hairs from abnormal sites is justifiable because of the psychic benefit rendered to the patient (5). Judgment must be exer-

cised to determine which patients should be subjected to high frequency epilation and which should be advised to use the time-honored methods of shaving, manual epilation, bleaching, cutting or waxes. Any or all of these methods are only temporary and cannot replace the faster, more efficient and permanent high frequency current. Electrolysis and the high frequency current are the most satisfactory methods. The former is slower and pain may be less intense, although the needle remains in longer and there is a greater regrowth of hair. Scars may occur with both methods, but with care they can be avoided. Less desirable procedures are the monopolar needle diathermy and vacuum tube diathermy. Good results may be obtained, however, with these machines.

Other methods of removing hair are mentioned only to be labeled as useless and, in some cases, actually harmful. Estrogens, in the form of estrone and alpha-estradiol, have proved to have no effect on hair growth when applied locally in hypertrichosis (6). Two European workers (7, 8) have reported that close-range fractionated roentgen therapy with the Chaoul apparatus resulted in permanent epilation. Knierer (7) followed his patients for less than three years. Porta's patients were observed for ten years (8). Cipollaro and Einhorn (9), however, representing the Council of Physical Medicine of the American Medical Association, in 1947 restated the opinion expressed in 1929 by the Association's Bureau of Investigation (10): "It is now the consensus of dermatologists and roentgenologists that superfluous hair cannot be permanently removed with radiation of any kind, regardless of technic, without permanent injury to the skin." This conclusion still being the opinion of the Council, these authors (9) maintain that no method for removing hair with roentgen rays should be approved, and add: "The immediate results of radiotherapy for hypertrichosis are always good. It takes months or years for radiodermatitis to develop and it takes even longer for the keratoses, ulcerations, malignant growths and death to occur." Illustrative cases bear out their conclusions, and cases reported by Cleveland (11) supply additional evidence of the inadvisability of treating hirsutism with roentgen rays. I have seen 2 patients with radiodermatitis of the chin treated by the Tricho system (an x-ray unit).

A warning against the possible use at some future date of radioisotopes for removing hair was also issued (9) by the Council in the statement. "There is no question whatever in the minds of physicians that radioactive isotopes can cause permanent defluvium of the hair but at the same time can so damage the skin that all the changes associated with a radiodermatitis, including malignant changes, will be manifested."

As stated previously, requests for treatment are being received with in-

creasing frequency. No doubt, this is partly due to more attention to appearances by both sexes. The not-so-young widow, about to sally forth to the matrimonial market, is frequently a patient, as are also older women who try to minimize their age for professional or social reasons. There is no indication that tumors of the adrenals or ovaries, the most usual endocrine factors responsible for hirsutism, occur any more frequently than formerly, or that the number of hidden endocrine disturbances is on the increase, but hypertrichosis seems to attract more attention and to be considered more objectionable than it was formerly.

A somewhat unusual case of hirsutism of male distribution occurring in a woman following a skull fracture has been attributed by Epstein (12) to irritation of the anterior hypophysis, stimulation of the adrenal cortex, and the production of androsterone. Removal of the hair by a high frequency spark-gap machine would be justified in such a case.

Another interesting and possible cause for hirsutism may be modern drug therapy. In 22 of 27 children from 3 to 14 years of age hypertrichosis developed during treatment of miliary and meningeal tuberculosis with streptomycin (13). Independent of sex, hair began to grow gradually after six to eight weeks of treatment, first on the upper side of the legs, then on most of the body (except the neck, elbows, knees and gluteal region). The length was from 1 to 3 cm. and the growth persisted after cessation of treatment. Hypertrichosis may also be anticipated following treatment with adrenal preparations. The author has observed one case in which it developed after the use of cortisone. Ragan (14) has called attention to the fact that the administration of ACTH or cortisone can be expected to produce the effects of naturally occurring hyperadrenocorticism, one of which is hirsutism.

SUMMARY AND CONCLUSIONS

A description is given of the author's technic for removing hair with the high frequency spark-gap machine at a rate of 40 to 50 hairs in five minutes, including his method of marking the needle with polyethylene tubing so that insertion can be made to the correct depth in the follicle. An explanation is offered for the increase in the number of patients who present themselves for treatment.

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CHANGING CONCEPTS IN THYROID PHYSIOLOGY AND THERAPY*

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IT HAS been a great honor and privilege to be president of the American Goiter Association during the past year. I have tried to serve in a manner consistent with the high standard established by the many outstanding men who have preceded me in this office.

The close cooperation of all the members of the Association, particularly of the various committees, has been very gratifying. I should like to mention briefly one matter which is vitally concerned with the future of this organization. Two groups have grown up in the American Goiter Association which for simplicity may be called the older group and the younger group. The older group consists of individuals who became members early in its development. The American Association for the Study of Goiter (the original name of this organization) was founded in 1923 by 26 men, most of whom were surgeons, although there were a few internists among the group, such as Henry Plummer, who introduced iodine in the treatment of toxic goiter.

With rapid developments in our knowledge of the thyroid, there came into the Association an active and energetic group of young investigators. The older group felt that the younger group was pushing them out, and the younger group felt that the older group had served its purpose and should step aside.

A similar situation exists in most societies. Most young men are restless and wish to see changes occur rapidly. The restless inquiring spirit of youth is very important and must be fostered and encouraged. At the same time it is very important for younger individuals to profit by the experience and advice of older people.

In my committee appointments during the past year I have made every effort to bring these two groups together. All of us have a common goal and are interested in developing as rapidly as possible our knowledge of the thyroid in health and disease. The harmony that has existed at this meeting appears to indicate that the two groups are working together in a satisfactory manner.

I am not going to talk very long this afternoon. As a matter of fact, I

* *Presidential Address* given at the Annual Meeting of the American Goiter Association, Chase Hotel, St. Louis, Missouri, May 2, 1952.

think most presidential addresses should be abolished. The president should see that the meeting runs smoothly, that the various committees function properly and that as many men as possible preside at the various sessions. However, since it is the custom of this organization for the president to say something, it occurred to me that a few reflections on thyroid physiology and therapy might be appropriate.

Developments have occurred at a very rapid rate just as they have in most fields of medicine and in various other fields of human endeavor. When this organization was started, there was a tendency to think of the thyroid in terms of this gland alone. We are now thinking of it in relation to other glands of internal secretion such as the pituitary and the adrenals and of its relationship to the hypothalamus and to the body as a whole.

We have learned a great deal about the nature of the thyroid secretion, although the more we learn, the more we realize how little we actually know. The more we learn, the closer we get to the mechanisms involved in normal thyroid physiology, to the manner in which they are disturbed in disease, and to methods of attack on the disordered function.

THE THYROXINE MOLECULE

In 1935 Thompson *et al.* (1) reported some studies on the physiologic effects of alterations in the thyroxine molecule. Observations in man on the calorigenic action of diiodotyrosine, thyronine, 3,5-*dl*-diiodothyronine and N-acetyl thyroxine suggested that the amino group, the diphenyl ether group and all four atoms of iodine were essential for the maximum effect of thyroxine. In patients with myxedema 3,5-*dl*-diiodothyronine had a definite calorigenic action which was much less than that of thyroxine. The maximum effect of a single dose occurred in one to two days compared with three to ten days following the administration of a single dose of thyroxine, and the effect wore off in seven to eight days compared with seventy to eighty days in the case of thyroxine.

Thompson *et al.* also reported that great changes in the basal metabolism in patients with myxedema might be produced by dinitrophenol without any alteration in the symptoms of myxedema. These observations suggested that there may be different types of altered metabolism that cannot be differentiated by changes in the rate of oxidation alone. Since these findings were reported, the physiologic effects of various other analogues of thyroxine have been observed.

Following these studies which were made with racemic thyroxine, many reports appeared which indicated that, *in vivo*, thyroxine is secreted in the levorotatory form and that the levorotatory isomer possesses most of the activity, the dextrorotatory form having only slight effect or no effect at

all. If this conclusion is correct, it would mean that our original figures for the rate of formation of thyroxine by the normal thyroid gland and for the amount of thyroxine in the body of a normal man outside of the thyroid gland (2) would have to be cut approximately in half. This would mean that the normal thyroid produces its hormone in terms of *l*-thyroxine at the rate of approximately $\frac{1}{6}$ mg. per day instead of $\frac{1}{3}$ mg. per day and that there are 5 to 7 mg. of thyroxine in the body of a normal man outside of the thyroid gland instead of 10 to 14 mg. However, the whole problem of the nature of the thyroid secretion and the rate at which the hormone is formed is in a state of flux, as indicated by the very interesting observations on analogues of thyroxine presented this morning by Lerman, Harington and Means (3).

A great deal of attention is now being focused on the physiologic activity of 3:5:3′-*l*-triiodothyronine. Preliminary observations on this compound have been reported by Gross and Pitt-Rivers (4, 5). It appears to be three to five times as effective as *l*-thyroxine. In a patient with myxedema with a basal metabolism of approximately −40 per cent, a single dose exerts its maximum effect on oxygen consumption within a period of eight to twenty-four hours, and the basal metabolism returns to a level of −40 per cent in about eight days (6), compared with periods of three to ten days and seventy to eighty days, respectively, for *l*-thyroxine. It is said to cause all of the manifestations of myxedema to disappear.

Conclusions concerning the relative calorigenic effects of triiodothyronine and thyroxine must be drawn with caution and should be based on the minimum amount required to maintain the basal metabolism of patients with myxedema at the normal level. Data based upon the total calorigenic effects of single doses may lead to different conclusions because of the much more prolonged effect of *l*-thyroxine.

Observations on the physiologic effects of triiodothyronine have opened up a new phase of investigation on the nature of the thyroid hormone. It would be unwise to draw any far-reaching conclusions concerning the significance of these studies until more data are available.

It has been suggested that thyroxine may be converted into triiodothyronine in the various tissues of the body. The possibility of the secretion of an altered hormone by the thyroid gland has also been suggested, as it has from time to time in the past. Triiodothyronine has been recovered from the thyroid gland and from the blood, but there are not enough data available at the present time to indicate whether or not the thyroid hormone is secreted in this form.

These observations make it necessary to carry out further investigations on the following problems:

1. The nature of the thyroid hormone and the form in which it is secreted.
2. The question of the secretion of altered types of the hormone.
3. The mechanism by which the thyroid hormone exerts its physiologic effects in tissues.

It is hoped that more information on these problems will be forthcoming at the 1953 meeting of the American Goiter Association.

It has been known for a long time that there are many factors in the body besides the thyroid hormone which influence total metabolism. The calorigenic effects of triiodothyronine, diiodothyronine and dinitrophenol emphasize this fact, as well as the importance of caution in drawing conclusions concerning the nature of alterations in total metabolism. This fact also has an important bearing on the interpretation of the nature of the low basal metabolism in certain patients who do not appear to have hypothyroidism.

An interesting related problem is the difference in the manifestations of hypothyroidism between the primary type and the type that is secondary to hypopituitarism. Patients with the secondary type have little or none of the characteristic edema, they do not gain weight and their skin is soft and delicate rather than dry and scaly. Both types of patients have hypothyroidism, but the clinical manifestations are different.

In patients with the primary type, the administration of desiccated thyroid eliminates all of the manifestations of the disease, but in patients with the secondary type the administration of desiccated thyroid corrects only some of the patients' symptoms. Those that are not corrected are presumably related to other glandular deficiencies secondary to the hypopituitarism. It has been suggested that the absence of stimulation of the adrenal cortex by the adrenocorticotropic hormone may account for some of the altered manifestations of hypothyroidism in the secondary type.

Other interesting clinical observations on patients with myxedema that require explanation are the following:

1. The characteristic manifestations of primary hypothyroidism do not appear until the basal metabolism has dropped to a level of between -20 and -25 per cent.
2. There is a considerable degree of lag between clinical changes and metabolic changes. Following the intravenous administration of a single dose of 10 mg. of thyroxine to a patient with complete myxedema, the period of rising basal metabolism is often associated with symptoms of intoxication, whereas the period of falling metabolism may be characterized by clinical improvement. The typical myxedematous state usually does not reappear for several months after

the basal metabolism has reached its original level of approximately −40 per cent, and the full-blown picture may not develop for several years after the low level of metabolism has returned.

THE PROTEIN CONTENT OF THE CEREBROSPINAL FLUID

I should like to call your attention to observations which were made several years ago on variations in the concentration of protein in the cerebrospinal fluid with variations in thyroid function (7, 8). These observations may be summarized as follows:

1. In patients with myxedema, the concentration of protein in cerebrospinal fluid is usually high and drops to within normal limits when the basal metabolism is restored to normal by the administration of desiccated thyroid.
2. In individual patients with myxedema, a very striking correlation may be observed between the level of basal metabolism and the concentration of protein in the cerebrospinal fluid.
3. In patients with hyperthyroidism, the concentration is low and rises to normal when the hyperthyroidism is eliminated by appropriate therapy.

These observations appear to be related to the increased deposition of protein in body fluids in patients with myxedema and the decreased concentration in patients with hyperthyroidism.

THE THYROID-STIMULATING HORMONE

Clinical and pathologic findings in patients with hypopituitarism and hyperpituitarism have indicated for a long time that a close relationship exists between the anterior lobe of the pituitary and the thyroid. In 1935 and subsequent years Thompson *et al.* (1, 9, 10) reported observations on the thyroid-stimulating effect of crude preparations of thyrotropic hormone. These observations may be summarized as follows:

1. In a large percentage of patients with any thyroid tissue capable of being stimulated, there was increased activity of the thyroid following intramuscular administration of thyrotropic hormone.
2. In patients with normal thyroid function, a syndrome resembling exophthalmic goiter was induced temporarily. However, we did not observe the development of exophthalmos in any of them, although it has been reported in experimental animals during the administration of thyrotropic hormone.
3. Nodular nontoxic goiter was converted temporarily into nodular toxic goiter.
4. Exacerbations of varying degrees of severity were induced in patients with exophthalmic goiter.

5. In patients with a moderate depression of thyroid function, the basal metabolism was raised temporarily to a normal or to a hyperthyroid level.
6. The increase in basal metabolism was always temporary, in spite of prolonged administration of the hormone.
7. The longest period of increase in basal metabolism which we observed was nine weeks, even though the hormone was continued for a much longer period of time.
8. In patients with normal levels of basal metabolism before administration of thyrotropic hormone, the basal metabolism sometimes dropped to a subnormal level following the disappearance of the increase in basal metabolism. The low level of metabolism was associated with a decrease in the size of the thyroid gland.
9. In patients with exophthalmic goiter, the exacerbation in symptoms during administration of the hormone was sometimes followed by a complete remission and disappearance of all of the manifestations of hyperthyroidism.
10. In patients with primary hypothyroidism and no thyroid tissue capable of function, the administration of thyrotropic hormone produced no effect on the basal metabolism.

EXOPHTHALMOS

The cause of exophthalmos in exophthalmic goiter has an important bearing on the nature of this disease. Recently Dobyns (11) has claimed that he has been able to isolate a substance from the pituitary which induces exophthalmos but has very little thyroid-stimulating effect. He has designated this material as EPS (exophthalmos producing substance). He thinks the stimulation of the thyroid and the production of exophthalmos may be induced by two different substances from the anterior lobe of the pituitary.

These observations need confirmation in various laboratories. If correct, they would explain certain phases of the development of exophthalmos which have been noted clinically for a long period of time, such as the following:

1. Exophthalmos may occasionally precede the development of hyperthyroidism by several months.
2. Exophthalmos may appear or increase when the basal metabolism has been reduced to a normal or a subnormal level by appropriate treatment.
3. Exophthalmos may be the only manifestation of the exophthalmic goiter syndrome that ever appears.

THE PRESENT STATUS OF THE TREATMENT OF TOXIC GOITER

At the 1951 meeting of this Association I presented a paper in which was given what appeared to be the consensus on the treatment of toxic goiter at that time. Very little modification in this point of view has occurred during the past year.

It has become increasingly apparent that, in patients who have nodular goiter with hyperthyroidism, the thyroid gland does not take up enough radioactive iodine to make the use of this substance an effective method of treatment. Subtotal thyroidectomy should be performed in all patients with hyperthyroidism associated with nodular goiter, unless there is some medical contraindication to operative procedures.

The search continues for a satisfactory medical treatment of toxic goiter. Most of our therapeutic attacks have been on the thyroid gland itself. Operative procedures, antithyroid drugs and radioactive iodine exert their effects directly through the thyroid.

It is surprising that in exophthalmic goiter the removal of two-thirds to seven-eighths of the thyroid gland produces a cure in as large a percentage of patients as it does. It seems probable that the cause of the disease is still active in many of the patients at the time of operation. In some patients the cause of the disease acts with great intensity over a long period of time. Two or three subtotal thyroidectomies followed by three or four doses of radioactive iodine may fail to effect a complete cure. In such patients the persistence of the disease can be explained by persistence of the cause. If this is true, it is difficult to understand why an apparent cure is produced in such a high percentage of patients by a so-called subtotal thyroidectomy.

Irradiation of the pituitary has been carried out in a small series of patients with exophthalmic goiter and nodular toxic goiter, and in some of them a complete remission has been produced. However, the percentage of remissions is too low to warrant the routine use of this procedure.

A great deal of evidence has accumulated to indicate that exophthalmic goiter develops as the result of a complex chain of events involving the thyroid, the anterior lobe of the pituitary and the hypothalamus. It is very important that intensive studies be carried out to determine the cause of this and other types of hyperthyroidism, so that medical treatment in the future may be directed at the cause, and our goal of developing a satisfactory form of medical therapy may be achieved.

SUMMARY

These brief remarks deal with only a few of the problems which confront the thyroid investigator at the present time. Many other interesting aspects of thyroid physiology and therapy can not be discussed because

of the limitation of time. It is hoped that these reflections may be of some interest to those concerned with unraveling the mysteries of thyroid function in health and disease.

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Letter to the Editor

ALLO-PREGNANE-3(β), 20(β)-DIOL AS A SUBSTITUTE STANDARD FOR PREGNANE-3(α), 20(α)-DIOL IN THE DETERMINATION OF URINARY PREGNANEDIOL

To the Editor:

The use of pregnane-3(α),20(α)-diol as a standard in the colorimetric determination of urinary pregnanediol in clinical laboratories presents a practical problem. Pure pregnane-3(α),20(α)-diol is not always available for the increasing number of laboratories which are instituting this estimation. In addition, the preparation of the pure compound is laborious. Haslam and Klyne (1) suggest that *allo*-pregnane-3(β),20(β)-diol may serve as a substitute standard, since they demonstrated that this synthetic product which is commercially available, has absorption characteristics similar to pregnane-3(α),20(α)-diol.

The present communication confirms the findings of Haslam and Klyne. The absorption spectra that we have obtained on the Beckman spectrophotometer are illustrated in Figure 1. It will be seen that the curves are similar except for differences at 375, 400, 420 and 430 mμ. We repeated our observations on a Cenco-Sheard spectrophotelometer, since most of the measurements in our laboratory are made on this instrument. Figure 2 reveals maximum absorption for *allo*-pregnane-3(β),20(β)-diol occurring at 420 mμ and maximum absorption for pregnane-3(α),20(α)-diol at 430 mμ. Differences similar to those observed on the Beckman spectrophotometer were noted with the Cenco-Sheard instrument.

To determine whether there was a constant relationship between percentage transmission and concentration, 10 to 100 gamma of each compound[1] was dissolved in 5 cc. of concentrated sulfuric acid, and the percentage transmissions measured at their respective maxima (Fig. 3). The readings were almost identical.

Since the absorption spectra of the two compounds are similar and since the relationships between transmission and concentration are identical at

[1] The *allo*-pregnane-3(β),20(β)-diol was made available for this study through Dr. K. W. Thompson, Organon, Inc., Orange, N.J.

The pregnane-3(α),20(α)-diol was provided by Dr. A. S. Cook, Ayerst, McKenna and Harrison, Ltd., Montreal, Canada.

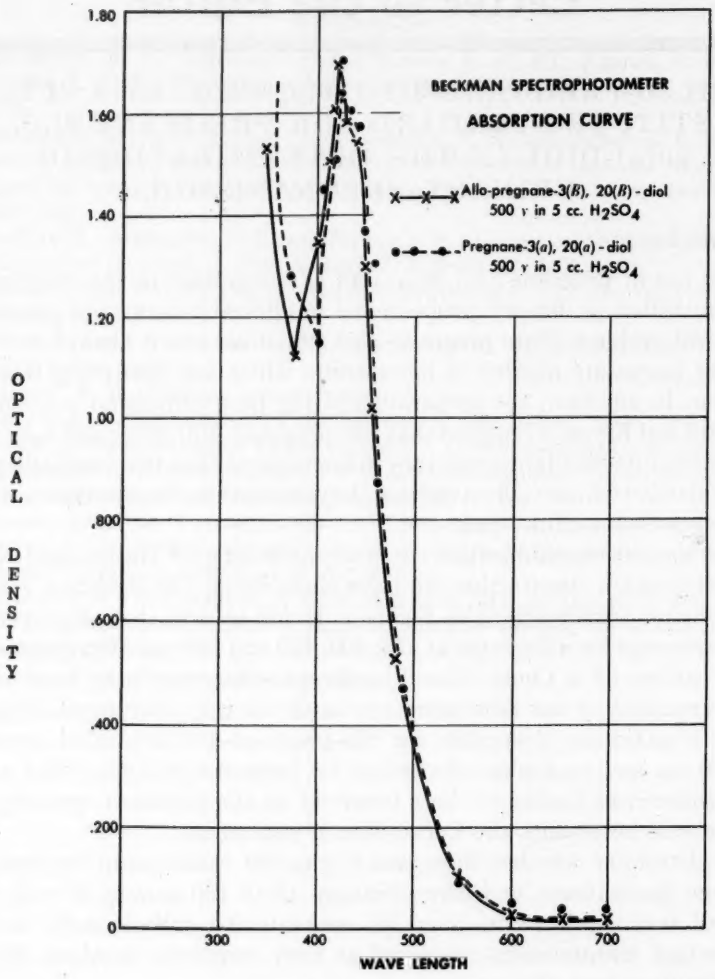
BECKMAN SPECTROPHOTOMETER
ABSORPTION CURVE
Allo-pregnane-3(B), 20(B) - diol
500 γ in 5 cc. H2SO4
Pregnane-3(a), 20(a) - diol
500 γ in 5 cc. H2SO4
OPTICAL DENSITY
1.80
1.60
1.40
1.20
1.00
.800
.600
.400
.200
0
300
400
500
600
700
WAVE LENGTH

FIGURE 1

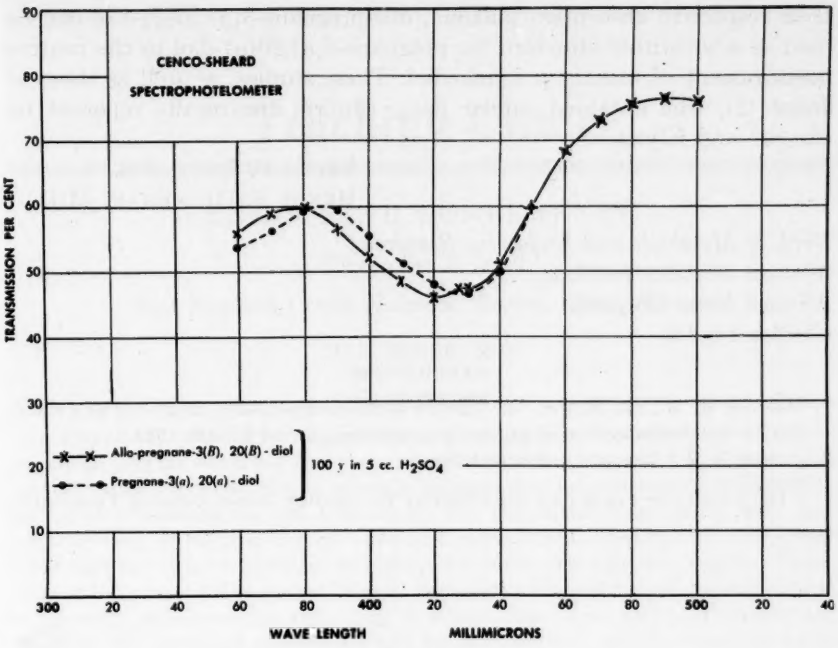

FIGURE 2

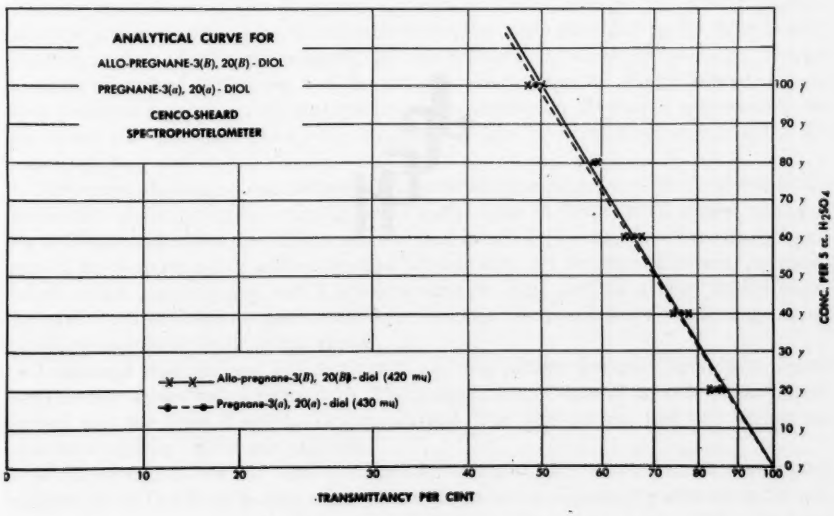

FIGURE 3

their respective absorption maxima, *allo*-pregnane-3(β),20(β)-diol can be used as a substitute standard for pregnane-3(α),20(α)-diol in the routine measurement of urinary pregnanediol. These studies, as well as those of Jailer (2), who obtained similar data, confirm the results reported by Haslam and Klyne.

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Teaching Clinic

ENDOCRINE CONFERENCE

November 3, 1952

New England Center Hospital, Boston, Massachusetts

M. S. RABEN, M.D.

Case history

A 42-year-old, married woman, mother of three children, was referred to this clinic because of a goiter which had first been noticed three months earlier. A year before this visit, following a five-year history of cough, a diagnosis of bronchiectasis was made with the aid of a bronchogram, and a lobectomy was performed. Nine months later, at a follow-up visit, an enlarged thyroid was noted, and at about the same time, the patient noted that her eyes were swollen and puffy, her throat felt tight and that her "voice sounded like gravel." The patient thought her hands were swollen, and there seemed to be muscular stiffness of the arms, legs, shoulders, and fingers and pain between the shoulders. The head and ears felt blocked, the skin was dry, and the fingernails broke easily. She felt "cold all the time," and very tired in the daytime; she slept poorly, had heart burn, had gained ten pounds in the three months before she was seen, and thought that her skin had become yellow.

The physical examination confirmed the abnormalities noted by the patient, particularly the yellowish puffy face, slow coarse voice, dry scaly skin, and goiter. The thyroid was irregularly and diffusely enlarged and was estimated to weigh 60–80 grams. The enlargement involved both lobes and the isthmus, and numerous poorly defined nodules were visible and palpable. No thrill or bruit was detectable. The pulse rate was 60; the heart beat was quiet and the rhythm regular. No other abnormalities were noted in the examination.

The serum cholesterol concentration was 405 milligrams per cent. Radioactive iodine studies showed a prompt accumulation of radioiodine in the thyroid region, amounting to 20 per cent of the dose in one and one-half hours. By twenty-four hours only 7 per cent of the dose remained and at ninety-six hours only 2.6 per cent. The serum protein-bound iodine concentration was 9.6 micrograms per cent and the urinary iodine excretion was 1,173 micrograms per twenty-four hours or nearly ten times the average excretion of normal individuals in this region.

Treatment with thyroid was instituted, and the patient was seen again after treatment for five weeks with 3 grains daily. The symptoms and signs of myxedema had disappeared, and the thyroid was no longer enlarged. The isthmus was just visible, but the lobes were neither visible nor palpable.

Medication with thyroid was continued for a total of three months. Six weeks after stopping there had been no recurrence of the symptoms or signs of myxedema and no return of thyroid enlargment. The serum cholesterol concentration was 219 milligrams

per cent, the serum protein-bound iodine 14.8 micrograms per cent, and the total serum iodine 27.2 micrograms per cent. The radioiodine study showed an uptake of 14 per cent in two hours and 13 per cent in twenty-four hours; at the end of eight days 11 per cent of the dose remained in the thyroid gland, presumably in an organically bound form.

Discussion

E. B. Astwood: The concurrent appearance of a goiter and signs and symptoms of myxedema with a rapid disappearance of the goiter following thyroid administration can be readily explained only by invoking an agent which interferes with thyroid function. Prolonged interference with thyroid hormone formation and resultant depletion of the store of hormone in the gland results in myxedema; the consequent excessive output of thyrotropin by the pituitary results in the development of goiter. Antithyroid agents which prevent the organic binding of iodine could be responsible. Among the thiocarbonamides, *l*-5-vinyl-2-thio-oxazolidone occurs in edible plants and is therefore a potential cause of this syndrome. Such chemotherapeutic agents as *p*-aminosalicylic acid and resorcinol can be responsible, and a number of related substances with anti-peroxidase activity are also potential agents. Aminothiazole produced these effects on workers in a sulfonamide factory in France.

The group of anions which impair the iodide-concentrating power of the thyroid may also induce this clinical picture. Thiocyanate is the best known of this group, but more recently perchlorate, nitrate and others have been shown to behave in the same way. Iodine would be a more efficacious treatment for thiocyanate goiter than it would for goiter caused by an antithyroid drug, whereas thyroid is effective therapy in either case.

Iodine must be placed in a category by itself in regard to the thyroid. Of the complex relationship between iodine and the thyroid, the effect which is most relevant to this discussion is the "antithyroid" action of high concentrations of iodine, an effect first demonstrated several years ago by Wolff and Chaikoff. Rapid elevation of the blood iodide concentration, and particularly of the thyroid iodide, inhibits the organic binding of iodine.

It is tempting to explain today's case in terms of this phenomenon, and full confidence in this explanation is diminished only by the fact that the experimental work to date has indicated that it is a temporary phenomenon, a matter of hours or days, far too brief to result in myxedema. It is conceivable, however, that under certain circumstances the inhibition could persist as long as the iodide concentration was high, and thus produce a clinical effect. There are scattered reports, in both the medical and the veterinary literature, of goitrous offspring born to mothers fed iodine during pregnancy, which might be explained this way.

This patient had a bronchogram one year before she was first seen here, and the iodized oil (Lipiodol) was still visible by x-ray examination. She had myxedema and a goiter for at least three months before being seen. Tracer studies indicated that the radioactive iodine uptake was of the type obtained with full antithyroid therapy. A maximum count was obtained in the early hours after I^{131} administration, and the radioactivity in the neck decreased rapidly thereafter. The rapid rise is due to the concentration of iodide ion in the thyroid and the equilibrium of the I^{131} with this iodide; the fall is due to urinary excretion. The maximum count, 20 per cent of the dose, is high for an iodide count and is a value which would be obtained in a hyperthyroid or thyrotropin-stimulated gland. The high count shows too that the iodide concentration at that time was not sufficient to depress the iodide-concentrating mechanism markedly. The high serum protein-bound iodine may be due to the radio-opaque oil or its degradation products in the blood, and the large excretion of iodine in the urine and the high serum iodide concentration may be attributable to the continuous liberation of inorganic iodine during the slow metabolism of the iodized oil.

I might add that the history and findings in this case are unique in our experience and it seems warranted to offer an equally unusual interpretation.

M. S. RABEN: In this unusual situation, one might obtain relief of both myxedema and goiter by the administration of thiocyanate. The interference with the iodide-concentrating mechanism would diminish the concentration of iodide in the thyroid, thus relieving the block to hormone formation.

S. PROGER: Some years ago, we saw myxedema develop in a patient who was receiving iodine therapy for hyperthyroidism. Although a rare occurrence, perhaps Dr. Astwood's interpretation of today's case is also applicable to the other.

S. J. THANNHAUSER: Despite the logic of Astwood's explanation, I do not understand why, if he is right, we do not see such cases more often.

E. B. ASTWOOD: Perhaps the conditions necessary to produce such prolonged thyroid inhibition with iodine are difficult to achieve and therefore only rarely occur. It is possible, too, that brief effects of this type occur more often, but that escape from the inhibition takes place before myxedema develops.

We see many simple goiters without myxedema which disappear with thyroid therapy, and in practically none of these cases do we discover a cause for the goiter. Perhaps some of these goiters occurred in this way.

W. P. VANDERLAAN: Whereas infection and malignancy are the important destructive agents for many organs, the thyroid seems to be peculiarly susceptible to chemical attack.

M. M. STANLEY: Perhaps it is just that more is known about the thyroid.

E. B. ASTWOOD: The parsimony of nature seems to disappear when it comes to estrogenic and to antithyroid compounds.

I. N. ROSENBERG: The existence of a large variety of antithyroid compounds is understandable if one believes that, in the thyroid, iodide burns in a flame fanned by peroxidase.

J. WOLFF: The blocking action of iodine is difficult to explain. Its mechanism of operation seems particularly complex since, as Morton, Chaikoff, and Rosenfeld, and also Raben have shown, below the critical concentration of iodide required to stop organic binding, the amount of iodine bound (in an acute experiment at least) increases with increasing iodide concentration. One would like to explain the effect of iodine on hyperthyroidism in terms of its blocking action, but such an explanation fails to account for the increased organic iodine content in the iodine-treated hyperthyroid gland. The clinical implication of the "antithyroid" action of iodine is nicely demonstrated in the case which has been presented here, and I am glad of the opportunity to see this patient.

Editorial

JOURNAL OF CLINICAL ENDOCRINOLOGY
AND METABOLISM

Publications Committee: Warren O. Nelson (Chairman), Edward W. Dempsey, Roy O. Greep, Roy G. Hoskins, E. Perry McCullagh, Willard O. Thompson.

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THE PSYCHOSOMATIC ASPECTS OF THYROTOXICOSIS

THE belief that thyrotoxicosis represents a constitutional disorder rather than merely a disease of the thyroid gland has persisted from the time of Marie (1883), who emphasized the nervous symptoms and, indeed, regarded the whole syndrome as of nervous origin (1). Crile (2) has recently remarked that the symptoms of "neurocirculatory asthenia" sometimes simulate hyperthyroidism so closely as to lead to surgical thyroidectomy. He agrees with Bleuler that psychosis at times presents symptomatology that is very difficult to differentiate from hyperthyroidism. The importance of nervous strain in precipitating the disorder has often been emphasized. On the other hand, schizophrenics, who have hit upon a mechanism for short-circuiting their emotional turmoil, are only rarely afflicted with Graves' disease. If, however, one takes the position that the disease is fundamentally a manifestation of "nervous trauma," the terms but not the essence of the etiology are merely shifted to new ground. Many people are subjected to severe nervous strain but in only a relatively small proportion of them does thyrotoxicosis develop. The basic question, therefore, is what makes the candidate for the disease vulnerable to the etiologic trauma? Does it occur generally, or is it restricted to people with some particular sort of background experience or of some peculiar personality type?

This aspect of the over-all problem has recently been considered by Ham, Alexander and Carmichael of the Chicago Institute for Psychoanalysis (3). In addition to a review of the relatively restricted psychodynamic literature on the subject, they present a study of 23 patients, 1 of whom was subjected to multiple psychoanalytic interviews.

They reach a conclusion that incorporates the findings of previous observers as well as their own results, namely, that the symptoms of thyrotoxicosis can indeed be understood as the patients' responses to early developmental experiences and that, although the experiences differ in details, they fall into a clearly definable pattern that is characteristic of the disease.

In the patients who were studied there appeared early in life an extreme feeling of personal insecurity, a strong sense of responsibility, and a tendency to "suffer in silence." The sense of insecurity gave rise to fear—often a fear of death—from which arose a strong impulsion to desperate and immature attempts to find security. In these precocious attempts they were frustrated by circumstances such as poverty, a rejecting attitude by parents, broken homes, and an unusual number of brothers and sisters of which the patients were commonly the eldest.

Thus was induced an excessive urge to accomplishment in order to control the given situation and to master the besetting fears. The result was a "pseudomaturity." A continued effort to mature, to grow up and be self sufficient, was judged to be the chronic emotional stimulus "which is so unique and important in this group of patients."

These frustrated, emotionally driven individuals show a marked tendency to overcompensate by taking care of others and especially, in women, by giving birth to and taking care of children. Because of the prematurity of their drive to mature, they are unable to reach a realistically successful solution of their problems. This failure leads to chronic anxiety that can not be allayed by relying on others for help, or by successful attainments. Thus arises a chronic stimulation of the organism to supply the energy needed for mature accomplishment and in this stimulation the thyroid shares. The failure of accomplishment itself and the neurotic mechanisms engendered, lead to accentuation of the anxiety and hence greater urge to performance. The vicious cycle thus arising, with "increasing disintegration of the normal controlling and limiting mechanisms" finally reaches expression in an attack of thyrotoxicosis. The disease is, then, in the authors' words, "a specific dynamic response, which is determined by the life-long personality development of the patient. In this response the thyroid gland is only one effective link in a chain of dynamic physiological events." In fairness to the Chicago investigators it should be added that, among the important frustrations, they list "unsuccessful premature attempts to identify with objects of defensive cravings." The psychodynamic implications of this mishap will be clearest to the psychoanalytically sophisticated but the theory should otherwise be intelligible even to confirmed organicists.

The authors are unnecessarily explicit in postulating that the anxiety sets up chronic overactivity in the diencephalon, which activates the anterior pituitary and causes overproduction of thyrotropic hormone. That exophthalmic goiter is actually due to overproduction of this hormone is open to doubt (4). There is little doubt, however, that nervous strain does, in some fashion, influence the thyroid; hence the Ham theory is tenable, whatever may be the actual mechanism of mediation.

A more serious question is whether the basic etiology of thyrotoxicosis is indeed, as postulated, a "continued effort to mature." In that case, the time-honored admonition, "first treat the cause," would come to bear in an interesting if not startling fashion. The theory should be further tested in a larger group of patients than have hitherto been studied from the psychodynamic point of view. Particularly, it should be incontrovertibly determined not only how consistently the foregoing psychodynamic constellation appears in the case histories of thyrotoxic patients but, of equal importance, how frequently it appears in those who escape the disease.

ROY G. HOSKINS, M.D.

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2. CRILE, GEORGE, JR: Practical Aspects of Thyroid Disease. Philadelphia, W. B. Saunders Co., 1949.
3. HAM, G. C.; ALEXANDER, F., and CARMICHAEL, H. T.: A psychosomatic theory of thyrotoxicosis, *Psychosomatic Med.* **13**: 18, 1951.
4. WERNER, S. C.; HAMILTON, H., and NEMETH, M.: Graves' disease: hyperthyroidism or hyperpituitarism?, *J. Clin. Endocrinol. & Metab.* **12**: 1561, 1952.

Questions and Answers

(Questions submitted at the 1952 Postgraduate Assembly of the Endocrine Society.)

Answers by John Eager Howard,
Baltimore, Md.

QUESTION: Discuss the reliability of increased calcium output on a (4+ days) restricted calcium diet (125 mg. daily) as an aid to accurate diagnosis of primary hyperparathyroidism.

ANSWER: Nearly all patients with proven hyperparathyroidism have had elevation of urinary calcium excretion above normal when studied on a restricted calcium diet as indicated in the question. It has been stated that hypercalciuria may not occur if renal insufficiency has supervened in primary hyperparathyroidism, but even under these circumstances, most hyperparathyroid subjects still exhibit hypercalciuria. One patient, from whom a parathyroid adenoma was removed with subsequent cure, excreted only 125 mg. of calcium in the urine per twenty-four hours when given a diet containing 125 mg. of calcium. The normal urinary calcium on such a diet is usually considered to be 100–125 mg. or below.

However, hypercalciuria does not necessarily mean hyperparathyroidism. With bed rest alone, especially if large skeletal areas are immobilized, patients may excrete 500–800 mg. of calcium per day in the urine, as may also patients with extensive paralysis from poliomyelitis. Acidosis results in hypercalciuria and patients with congenital hyperchloremic acidosis may excrete large quantities of calcium, even though the urine is apt to be alkaline. Patients with hypercalcemia from any cause (bone tumors, sarcoidosis, myeloma) excrete excessive calcium in urine and, in certain stages of their disease, patients with Cushing's syndrome do likewise, even in the absence of hypercalcemia.

Thus it may be concluded that though studies of quantitative urinary calcium may be helpful in the diagnosis of hyperparathyroidism, it is but one factor to be considered in the over-all clinical picture.

QUESTION: Is there sufficient evidence to justify mediastinal exploration in the following case? Markedly increased urinary calcium (550–660 mg. in 24 hours—4 tests), no polyuria; normal levels of serum calcium, phosphorus, alkaline phosphatase, nonprotein nitrogen, and protein; no evidence of other bone disease, multiple myelomas or severe nephritis; negative findings from exploration of the neck.

ANSWER: Not on the basis of the hypercalciuria alone. Patients with acidosis, and invividuals at bed rest, especially those with large areas immobilized, may excrete this much calcium in the urine. It seems extremely doubtful that, from hyperparathyroidism alone, a patient would excrete more than 500 mg. of calcium in the urine per day on a low calcium intake, without havlng some elevation of serum calcium and lowering of serum phosphorus levels.

QUESTION: Will steroid hormone therapy help in the healing of fracture?

ANSWER: The evidence for this in the human is very dubious, but I do not know of any work that will positively refute the statement that some hastening of fracture healing may be obtained with ovarian hormone therapy.

Answers by Rulon W. Rawson,
New York, N.Y.

QUESTION: Please discuss the use of I^{131} in thyroid crises.

ANSWER: Radioactive iodine has no place in the treatment of thyroid crisis. The maximum effect of radioactive iodine is not obtained for several weeks after administering it and, therefore, would have no effect in treating such a medical emergency.

QUESTION: What is the significance of low serum cholesterol values of 105 and 106 (determined in two different laboratories) in a patient with marked muscle weakness but no evidence of hyperthyroidism?

ANSWER: A low serum cholesterol value of 105 mg. per cent may be seen in patients with starvation and such patients might have marked muscle weakness. However, a careful search for increased thyroid hormone coming from a small hyperplastic thyroid, a hyperfunctioning adenoma, struma ovarii, or from a bottle (thyrotoxicosis factitia) should be undertaken. Often a serum protein-bound iodine determination or a tracer with radioactive iodine will reveal hyperthyroidism that is not evident with the more classic diagnostic studies.

QUESTION: How do you feel about thyroid adenomas and surgery from the point of view of potential malignancy? Should thyroid adenomas all be removed?

ANSWER: The incidence of cancer in single nodules of the thyroid has been variably reported as being between 10 and 24 per cent. If competent thyroid surgery is available, we would advocate the removal of single nodules of the thyroid—if for no other reason than to make a diagnosis.

QUESTION: If TSH, or the "exophthalmic factor," is responsible for the eye signs in Graves' disease, apparently due to overproduction of TSH, why don't all cases of myxedema or hypothyroidism have measurable exophthalmos?

ANSWER: It has not been proved that TSH is the exophthalmic factor; indeed, there is some evidence suggesting that these two hormone factors of the anterior pituitary may be separate. For instance, the exophthalmic factor, as demonstrated in the fundulus per unit of thyrotropic activity, is much greater in the sheep pituitary than in the bovine pituitary. If these factors are, however, the same, the fact that we do not see exophthalmos more frequently in myxedema or hypothyroidism suggests that there are other factors involved in exophthalmos than the so-called pituitary factor. Further study is indicated to elicit such other factors.

Answer by Henry F. Klinefelter,
Baltimore, Md.

QUESTION: If we rule out TSH stimulation as a cause of exophthalmos, what do you think of the possibility of direct stimulation of Müller's muscle by hypothalamines or sympathine?

ANSWER: Exophthalmos can be produced in lower animals by stimulation of the cervical sympathetic nerve trunk or by administration of thyroxine, sympathicomimetic substances or cyanides. These act by causing a contraction of Müller's orbital mscle. In man, however, Müller's muscle is very small, and different from the strong muscular cone found in lower animals. There is no evidence that stimulation of Müller's muscle is responsible for human exophthalmos.
Ref: Brunton, C.—*Physiol. Rev.* **29**: 260, 1949.

Answers by John McLeod,
New York, N.Y.

QUESTION: What climate would you recommend for the subfertile couple? Temperature? Altitude?

ANSWER: Although it is true that febrile states inhibit spermatogenesis in the human, and that high altitudes produce temporary sterility in sheep and certain other species, it does not follow that living in the Arctic regions or at an altitude approximating that of Death Valley will aid the subfebrile couple. It is neither feasible nor scientifically sound to recommend a "change of climate" for infertility, except perhaps that a change of environment (vacations, etc.) may induce the mental relaxation in the couple which often produces the desired result.

QUESTION: It has been said that the volume of the ejaculate is correlated very closely with the androgen level of the man. In other words, a low seminal volume, even with a good sperm concentration, indicates a level of androgen which is lower than optimal for the subject. Would you comment on your experience with this phenomenon.

ANSWER: In the "intact" individual, there is no sound evidence to indicate that there is a close correlation between ejaculate volume and androgen level.

Answers by Fred A. Simmons,
Boston, Mass.

QUESTION: What is the best treatment to induce ovulation?

ANSWER: There is no known treatment to induce ovulation which is acceptable to all. There are various reported successes, running the gamut from the administration of thyroid to x-ray treatment of the pituitary and ovaries.

The most reliable treatment which results in ovulation is the wedge-shaped surgical resection of both ovaries in patients suffering from polycystic ovaries usually associated with abnormal temperature curves and possibly obesity, hirsutism and amenorrhea (the Stein-Leventhal syndrome).

Another program followed by the event of ovulation is the administration of cyclic estrin and progestin. The first month the patient takes a daily dose of 1 mg. of stilbestrol orally for thirty days, followed by the daily administration of 10 mg. of progesterone sublingually for six days. The second month the dose of stilbestrol is lowered to 0.5 mg. daily for twenty-one days, followed by progesterone in the same dosage as previously. The third month the dose of stilbestrol is lowered to 0.25 mg. daily for twenty-one days, followed by the usual dosage of progesterone. This should induce cyclic menstrual flow but not necessarily ovulation. The ensuing month or two the patient may ovulate spontaneously.

QUESTION: For religious reasons sperm tests (examinations) are not practical with some patients, but the Sims-Huhner test would be. Is this test enough, in your opinion, to determine whether or not a man is fertile?

ANSWER: No. Under no circumstances is the Sims-Huhner test an adequate assay of male fertility. Very often a man with marked oligospermia or poor motility of the sperm will present the clinician with a fairly good result from the Sims-Huhner test in the cervical mucus, particularly if the wife is highly normal. On the other hand, given a wife with extensive endometriosis, nonpatent tubes, or anovulatory cycles, if the Sims-Huhner test reveals 25–50 actively motile sperm per high-power field, it is probably fair to say that this particular husband is within normal limits of fertility.

To overcome the "religious reasons," Dr. J. B. Doyle of Boston has devised the Doyle spoon which allows the collection of an uncontaminated ejaculate from the vagina directly post-coitally. I am told this has the sanction of the Church.

Under other circumstances, in certain faiths, permission can be obtained for the proper production of a semen specimen.

The Endocrine Society

35TH ANNUAL MEETING

MAY 28–30, 1953

Headquarters: Hotel Statler, New York, New York.

Registration: Everyone attending the meetings is required to register. By action of the Council, non-members will be charged a registration fee of $5 for any part of the scientific sessions.

Fellows, graduate students, residents, and interns will be admitted upon presentation of proper credentials certified by a department head or superintendent of a hospital and payment of $2 registration fee.

Members should present their current membership cards when registering. Admission will be by badge only.

The Scientific Sessions: Scientific Sessions on Thursday, May 28, will be held in the Penn Top Room and on Friday and Saturday, May 29 and 30, in the Grand Ballroom of the Hotel Statler. (The Joint Session with the American Diabetes Association will be held in the Hotel Commodore, Saturday, May 30, at 2:00 P.M.) Sessions will begin promptly on schedule. Presentation of papers at all sessions will be LIMITED TO TEN MINUTES and authors are requested to adhere strictly to this schedule. Manuscripts of all papers should be submitted to the presiding officer or to the Secretary-Treasurer at the end of the presentation.

Annual Dinner: The Annual Dinner of the Society will be held on Friday evening, May 29, at 7:30 o'clock in the Georgian Room, preceded by cocktails at 6:30 P.M. in the same room. Secure tickets at time of registration.

Local Arrangements: The Committee on Local Arrangements for the meeting:—Dr. Earl T. Engle (Chairman), Dr. Rulon W. Rawson, Dr. Sydney C. Werner, and Dr. Edward Rall.

Secretary-Treasurer: Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma.

PROGRAM

THURSDAY, MAY 28, 1953

8:30 A.M. Registration (*Hotel Statler*)

I. 9:30 A.M. GONADS, SEX HORMONES AND GONADOTROPINS (*Penn Top Room*)

Chairmen: Warren O. Nelson and C. L. Buxton

1. QUANTITATIVE ASPECTS OF REGRESSION OF PROSTATE AND UTERUS OF THE RAT FOLLOWING HORMONAL DEPRIVATION. *Roy Hertz* and *William W. Tullner* (by invitation).

2. THE PROLONGED ANABOLIC ACTIVITY OF CERTAIN TESTOSTERONE ESTERS IN MAN. *R. Palmer Howard, Edward C. Reifenstein, Jr., Claiborne L. Courtright* (by invitation) and *Henry H. Turner.*

3. ELASTIC FIBERS IN THE TUNICA PROPRIA OF NORMAL AND PATHOLOGICAL HUMAN TESTES. *F. A. de la Balze, G. E. Bur* (by invitation), *Clorinda Scarpa Smith* (by invitation) and *Juan Irazu* (by invitation).

4. THE EFFECTS ON THE TESTES OF SUPPRESSION OF ADRENAL HYPERPLASIA BY CORTISONE. *José Cara* (by invitation) and *Lawson Wilkins.*

5. THE HISTOLOGICAL RESPONSE OF THE GONADS OF INTACT IMMATURE MICE TO INJECTIONS OF GONADOTROPINS. *Helen Thomopoulou* (by invitation) and *Choh Hao Li.*

6. THE EFFECTS OF HOG PITUITARY GONADOTROPIN THERAPY AND SUBSEQUENT ANTIHORMONE FORMATION ON OVARIAN FUNCTION. *William O. Maddock, Ichiro Tokuyama* (by invitation), *Robert B. Leach* (by invitation) and *William R. Roy* (by invitation).

7. THE GONADOTROPHIC INHIBITING EFFECT OF AN AUGMENTOR EXTRACTED FROM THE URINE OF PREGNANT MARES. *Mia Parsonnet* (by invitation), *Herman Cohen* (by invitation), *Leonard Hirsch* (by invitation) and *Herbert S. Kupperman.*

8. THE EFFECT OF PROGESTERONE ON THE LACTIC DEHYDROGENASE-DPNH OXIDASE SYSTEM OF OVARIECTOMIZED RAT UTERI. *Arley T. Bever* (by invitation), *Joseph T. Velardo* (by invitation), and *Frederick L. Hisaw.*

9. THE EFFECT OF ESTROGEN PRETREATMENT ON AMINO ACID INCORPORATION IN SURVIVING RAT UTERI. *Gerald C. Mueller* (introduced by *E. S. Gordon*).

10. SOME OF THE HORMONES REQUIRED BY THE MAMMARY GLAND IN ITS DEVELOPMENT AND FUNCTION. *William R. Lyons, Choh Hao Li, Roger D. Cole* (by invitation) and *Ruth E. Johnson* (by invitation).

TO BE PRESENTED IF TIME PERMITS

11. INHIBITION BY ESTROGEN OF "COMPENSATORY" RENAL HYPERTROPHY IN THE RAT. *C. A. Schaffenburg* and *E. Perry McCullagh.*

12. HUMAN URINARY GONADOTROPHIN IN PREGNANCY. *R. A. Lyon, M. E. Simpson* and *H. M. Evans.*

13. THE INFLUENCE OF PHYSIOLOGIC DOSES OF THE SEX STEROID HORMONES ON THE SERUM LIPIDS AND LIPOPROTEINS IN MEN AND WOMEN. *S. J. Glass, H. Engelberg* (by invitation), *J. Gofman* (by invitation) and *H. B. Jones* (by invitation).

II. 2:00 P.M. ADRENALS AND ACTH (*Penn Top Room*)

Chairmen: Peter H. Forsham and Laurance W. Kinsell

14. SELECTIVE ARREST IN THE DEVELOPMENT OF ONE EXTREMITY BY TREATMENT WITH HYDROCORTISONE. *Hans Selye.*

15. Studies on Androgen Production by the Human Adrenal Cortex. *Paul L. Munson, John C. Laidlaw* (by invitation), *Frederick C. Goetz* (by invitation), *J. Hartwell Harrison* (by invitation) and *George W. Thorn.*

16. Importance of the Liver in Transformations of Administered Adrenosteroidal Compounds. *Stefan S. Fajans* (by invitation), *Lawrence H. Louis* (by invitation), *Holbrooke S. Seltzer* (by invitation) and *Jerome W. Conn.*

17. The Effect of Cortisone Upon the Fatty Liver and Kidney Changes of Choline Deficient Rats. *Elizabeth L. Hurd* (by invitation), *Florence K. Bass* (by invitation), *Arthur C. DeGraff* (by invitation), and *Herbert S. Kupperman.*

18. The Simultaneous Measurement of Exchangeable Sodium and Potassium Spaces in Man. *Walter L. Arons* (by invitation), *Robert J. Vanderlinde* (by invitation) and *Arthur K. Solomon* (by invitation) (introduced by *George W. Thorn*).

19. Water and Salt Metabolism During Acute Adrenal Insufficiency in Man. *Olof H. Pearson, Mortimer L. Mendelsohn* (by invitation) and *Charles D. West.*

20. The Anti-Anabolic Effect of Cortisone on the Anabolic Property of Intravenously Administered Serum Albumin. *Philip H. Henneman, A. P. Forbes, Eleanor F. Dempsey* (by invitation) and *Fuller Albright.*

21. Physiological Effects of Cortisone in Man. *Charles D. West, Olof H. Pearson* and *Attallah Kappas* (by invitation).

22. Functional Evidence in Man for the Existence of an Adrenal Growth Factor Distinct from ACTH. *G. W. Liddle* (by invitation), *A. P. Rinfret* (by invitation), *J. Richard* (by invitation), and *P. H. Forsham.*

23. Relationship of Renal Excretion of Biologically Active Adrenal Corticoids to Variations in Glomerular Filtration Rate. *Leon J. Marks* and *Alexander Leaf* (introduced by *N. B. Talbot*).

24. Influence of Stress on the Action of Cortisone. *K. E. Paschkis, A. Cantarow, A. M. DeAndiano, Jr., D. Epstein* (by invitation), *W. Abelove* (by invitation) and *E. Mazur* (by invitation).

TO BE PRESENTED IF TIME PERMITS

25. Studies on the Adrenal Cortical Response to Stress in Man. *Dalton Jenkins* (by invitation), *John C. Laidlaw* (by invitation), *William J. Reddy* (by invitation), *Lewis C. Mills* (by invitation) and *George W. Thorn.*

26. The Influence of Dietary Factors on the Hyaluronidase Spreading Reaction in Intact and Adrenalectomized Rats. *Edith M. Beck* (by invitation) and *Elaine P. Ralli.*

27. The Influence of Starvation and the Adrenal Upon Iron Storage in the Rat. *Diana F. Silber* (by invitation) and *Albert S. Gordon.*

FRIDAY, MAY 29, 1953

8:30 A.M. Registration

III. 9:00 A.M. STEROIDS AND BIOASSAY METHODS (*Grand Ballroom*)

Chairmen: Leo T. Samuels and Ira T. Nathanson

28. The Estrogenic Activity of Urinary Preparations: Studies of Ultrafiltration, Alcohol Precipitation, and Kaolin Adsorption. *H. M. Lloyd, A. J. Popert* and *E. F. Scowen* (introduced by *Fuller Albright*).

29. Bioassay of Adrenal Corticoids by Means of a Thymus Weight Response in Weanling Rats. *N. R. Stephenson* (introduced by *L. I. Pugsley*).

30. A Clinically Significant ACTH Assay Based upon Urinary 17-Hydroxycorticoid Excretion in Guinea Pigs. *G. W. Liddle* (by invitation), *D. Island* (by invitation), *M. Dimitman* (by invitation) and *P. H. Forsham.*

31. Fractionation by Countercurrent Distribution of Fluorometrically Measured Urinary Estrogens. *Claude J. Migeon* (introduced by *Lawson Wilkins*).

32. Metabolism of Isotopic Estrone in Human Pregnancy. *W. H. Pearlman, M. R. J. Pearlman* (by invitation) and *A. E. Rakoff.*

33. A New Method for Rapid Chromatographic Identification of Cortical Steroids: Application to Biological Fluids. *Maurice Pechet* (introduced by *Fuller Albright*).

34. The Detection of Pregnandiol and Pregnantriol in the Urine of Patients with Adrenocortical Hyperplasia. Suppression with Cortisone. *Alfred M. Bongiovanni* (by invitation) and *Lawson Wilkins.*

35. Effect of ACTH and Various Substrates on in Vitro Adrenal Cortical Steroid Synthesis. *Frederick G. Hofmann* (by invitation) and *Clarke Davison* (by invitation) (introduced by *Roy O. Greep*).

36. Further Studies on Cortisone Metabolism in Perfused Rat Liver. *O. Hechter, E. Caspi* (by invitation) and *H. Levy* (by invitation).

37. Fate and Excretion of Exogenously Administered Cortisone. *Don H. Nelson* (by invitation) and *George W. Calver.*

38. Disappearance of Progesterone from the Blood Following Intramuscular and Intravenous Injections of the Hormone. *R. L. Shoger* (by invitation) and *M. X. Zarrow.*

TO BE PRESENTED IF TIME PERMITS

39. A Standard ACTH-Adrenal Steroid Response Test. Evaluation in Children. *Vincent C. Kelley* and *Robert S. Ely* (introduced by *Leo T. Samuels*).

40. Peritoneal Fluid as a Site for the Study of Endocrine Effects on Blood Cells. *Jacques Padawer* (by invitation) and *Albert S. Gordon.*

41. Human Corticosterone Metabolism. (3) The Urinary Pettenkofer Chromogen Increased by Corticotropin or Corticosterone is not Dehydro-epiandrosterone. *William Q. Wolfson, Mioko Chambliss* (by invitation) and *William D. Robinson* (by invitation).

IV. 1:30 P.M. ANNUAL BUSINESS MEETING OF THE ENDOCRINE SOCIETY (*Grand Ballroom*)
(all members requested to attend promptly)

V. 2:30 P.M. THYROID (*Grand Ballroom*)
Chairmen: E. B. Astwood and Robert H. Williams

42. The Effects of Thyroxine and Triiodothyronine on Oxygen Utilization by Tissues in Vitro. *John G. Wiswell* and *Samuel P. Asper, Jr.*

43. Comparative Calorigenic Effect of *l*-Tri-Iodothyronine and *l*-thyroxin Given Intravenously to Myxedematous Patients. *Charles M. Blackburn* (by invitation), *William M. McConahey, F. Raymond Keating, Jr.*, and *A. Albert.*

44. A Unique Serum Iodine Component in Certain Patients with Thyroid Carcinoma. *Jacob Robbins* (by invitation), *J. E. Rall* and *Rulon W. Rawson.*

45. Effect of Concurrent "Dibenzyline" Administration on Thyroxine-Induced and Epinephrine-Induced Increased Oxygen Consumption. *Dorsey E. Holtkamp* and *Arthur E. Heming.*

46. Influence of Cortisone and Desoxycorticosterone upon the Renal and

THYROIDAL METABOLISM OF IODINE. *Sidney H. Ingbar* and *Bruce F. Chandler* (introduced by *Laurence H. Kyle*).

47. THE EFFECT OF SODIUM IODIDE, THYROXINE AND CORTISONE ON SERUM TSH LEVELS IN THYROIDECTOMIZED RATS. *Benjamin Simkin, Paul Starr* and *Charles Hancock* (by invitation).

48. THE INFLUENCE OF CORTISONE ON PITUITARY-THYROID INTERACTION IN NORMAL AND GOITROUS RATS. *S. A. D'Angelo, C. E. Stevens* (by invitation), *K. E. Paschkis* and *A. Cantarow.*

49. OBSERVATIONS ON THE CONTROL OF THYROTROPIN SECRETION OF INTRAOCULAR PITUITARY IMPLANTS IN HYPOPHYSECTOMIZED MICE. *Robert O. Scow* (by invitation) and *Monte A. Greer.*

50. THE DIAGNOSIS OF EQUIVOCAL HYPOTHYROIDISM USING THYROTROPHIC HORMONE (TSH). *Norman G. Schneeberg, William H. Perloff* and *Lester M. Levy.*

51. THE TOLERANCE OF PATIENTS WITH MYXEDEMA TO EXOGENOUS THYROID SUBSTANCE. *Richard Goldsmith* (by invitation) and *John B. Stanbury.*

TO BE PRESENTED IF TIME PERMITS

52. THE EFFECTS OF CORTISONE IN ACUTE DIFFUSE THYROIDITIS. *Robert M. Salassa* (introduced by *Randall G. Sprague*).

53. INVESTIGATION OF THE EXTRA-THYROIDAL EFFECTS OF THYROID STIMULATING HORMONE. *L. H. Kyle, W. C. Welham* (by invitation), *P. D. Doolan* (by invitation) and *M. Schaaf* (by invitation.)

54. BEHAVIOR CYCLES PRODUCED IN RATS BY THYROIDECTOMY, INJECTION OF I^{131} OR BY FEEDING SULFAMERAZINE. *Curt P. Richter, G. E. Seegar Jones* and *James W. Woods* (by invitation).

VI. ANNUAL DINNER

Paul Starr, President, Presiding

6:30 P.M. Cocktails (*Georgian Room*)
(Sponsored by the donors of the Awards of the Society: Ayerst, McKenna and Harrison; Ciba; Schering; and Squibb.)
Attendance limited to holders of dinner tickets.

7:30 P.M. Dinner (*Georgian Room*)

PRESIDENTIAL ADDRESS

Presentation of: The Squibb Institute for Medical Research Award.
The Ciba Award.
The Schering Fellowship in Endocrinology.
Lawson Wilkins,
Chairman of the Committee on Awards

SATURDAY, MAY 30, 1953

8:30 A.M. Registration

VII. 9:00 A.M. HYPOTHALAMUS, PARATHYROID, GROWTH HORMONE, PANCREAS (*Grand Ballroom*)

Chairmen: Francis D. W. Lukens and James H. Leathem

55. ENDOCRINE AND OTHER CLINICAL MANIFESTATIONS OF HYPOTHALAMIC DISEASE; A SURVEY OF 60 AUTOPSIED CASES. *Hans G. Bauer.*

56. Fractionation of Hypothalamus for Pituitary-Stimulating Activity. *M. A. Slusher* (by invitation) and *Sidney Roberts.*

57. Antidiuretic Hormone Antagonism. *George E. Miller* and *Charles E. Townsend* (introduced by *George F. Koepf*).

58. Idiopathic Hypercalciuria. *Fuller Albright, Philip Henneman, Patricia H. Benedict* and *Anne P. Forbes.*

59. The Effect of Parathyroid Extract on Ammonium-Chloride-Decalcified-Bone Matrix and on the Serum Levels of Polysaccharides; Preliminary in Vivo Studies in Parathyroidectomized Rats. *Claiborne L. Courtright* (by invitation), *M. R. Shetlar* (by invitation) and *Edward C. Reifenstein, Jr.*, in collaboration with *Harry B. Jones* and *R. C. McDougal.*

60. A Simple Test to Study Calcium Metabolism in Endocrine Disorders. *Herta Spencer, Isaac Lewin* and *Daniel Laszlo* (introduced by *Walter Fleischman*).

61. The Effect of "Pituitary Growth Hormone Preparations" in Human Subjects. A Summary of Quantitative Studies Extending over a Four Year Period. *Laurance W. Kinsell, Sheldon Margen, John W. Partridge, George D. Michaels, Harry E. Balch* and *John P. Jahn.*

62. Utilization of Labelled Glucose and Fructose by Liver Slices of Normal and Diabetic Rats. *Albert E. Renold, Frances B. Nesbett* and *A. Baird Hastings* (introduced by *George W. Thorn*).

63. Metabolic Observations Following Total Pancreatectomy. *M. A. Ogryzlo* (by invitation), *A. G. Gornall* (by invitation), *J. A. Dauphinee* (by invitation) and *D. J. MacKenzie* (by invitation) (introduced by *R. F. Farquharson*).

64. The Physiologic Consequences of Selective Destruction of the Alpha Cells of the Pancreatic Islets. *Sydney S. Lazarus* (by invitation), *Bruno W. Volk* (by invitation) and *Martin G. Goldner.*

65. Steroid Diabetes in Guinea Pigs and the Role of Insulin in the Adaptation to Cortisone Administration. *F. X. Hausberger* (introduced by *William H. Pearlman*).

TO BE PRESENTED IF TIME PERMITS

66. The Prediabetic Father. *W. P. U. Jackson* (introduced by *Fuller Albright*).

VIII. 2:00 P.M. JOINT SESSION WITH THE AMERICAN DIABETES ASSOCIATION (*Hotel Commodore*)

67. Growth Hormone as an Anti-insulin Factor. *Richard C. de Bodo* (by invitation).

68. Metabolic Effects of Crystalline Growth Hormone (Somatotropin) in Man. *Ephraim Shorr* (by invitation), *Anne C. Carter* (by invitation), *B. J. Kennedy* (by invitation) and *Richmond W. Smith, Jr.* (by invitation).

69. The Banting Memorial Lecture: An Interpretation of Diabetes in Light of its Pathology. *Shields Warren* (by invitation).

70. Effect of Cortisone on Carbohydrate Metabolism, Measured by the Glucose Assimilation Coefficient. *P. A. Bastenie* (by invitation), *J. R. M. Franckson* (by invitation) and *V. Conard* (by invitation) (introduced by *E. H. Rynearson*).

71. Studies in Experimental Diabetic Acidosis: Comparison of the Effect of Fructose and Glucose in the Initial Hours of Treatment. *Max Miller, J. R. Murphy* (by invitation), *J. W. Craig* (by invitation) and *Hiram Woodward, Jr.* (by invitation).

72. The Serum Polysaccharides in Diabetic Patients with and without Degenerative Vascular Disease. *Harold Rifkin, James Berkman* (by invitation) and *George Ross* (by invitation).

73. Spontaneous Diabetes in Dogs: an Account of Eight Cases. *Henry T. Ricketts.*

TO BE READ BY TITLE

Gonads, Sex Hormones and Gonadotropins

74. Study of the Morphological and Histochemical Changes Produced by Estrogens on Adult Human Testes. *F. A. de la Balze, R. E. Mancini* (by invitation), *G. E. Bur* (by invitation) and *Juan Irazu* (by invitation).

75. The Control of Functional Uterine Bleeding (Menometrorrhagia) by the Oral Administration of Progesterone and Methyltestosterone. *Meyer H. G. Blatt* (by invitation), *Hans Wiesbader* (by invitation), and *Herbert S. Kupperman.*

76. The Time of Ovulation in the Human Female. V. Trans-Vaginal Pelvioscopy: Correlation of Ovarian Status with the Basal Body Temperature Curve and Endometrial Biopsy. *A. R. Abarbanel.*

77. In Which Cases of Female and Male Sterility are Hormone Assays of Value? *L. I. Swaab.*

Adrenals

78. The Simultaneous Administration of ACTH and Cortisone or Hydrocortisone. *Joseph W. Goldzieher, Earl Baker* (by invitation) and *William B. Rawls* (by invitation).

79. Stimulation by Exogenous ACTH Administration of an Adrenocortical Adenoma Producing Cushing's Syndrome. *F. O. Kolb, J. H. Bruce* (by invitation), *G. W. Liddle* (by invitation), *G. M. Miller* (by invitation), and *P. H. Forsham.*

80. Differential Intramuscular Inactivation of Highly Purified ACTH Preparations in Man. *P. H. Forsham, G. W. Liddle* (by invitation), and *D. Bleifer* (by invitation).

81. Hepatic Phospholipid Metabolism and the Adrenal. *Franz Halberg, Cyrus P. Barnum* and *Halvor Vermund* (introduced by *Maurice B. Visscher*).

82. Some Effects of Compound F and DCA on Organic and Inorganic Metabolism in the Adrenalectomized Rhesus Monkey. *Ernest Knobil* (by invitation), *Frederick G. Hofmann* (by invitation) and *Roy O. Greep.*

83. Influence of Sex on the Excretion of an Acid Load by Adrenalectomized Rats. *Mary E. Dumm, Stephen H. Leslie* and *Bertram Laken* (by invitation).

84. Effect of Cold on Pituitary-Thyroid-Adrenal Relationships in the Guinea Pig. *C. E. Stevens* (by invitation), *S. A. D'Angelo, K. E. Paschkis, A. Cantarow* and *F. William Sunderman* (by invitation).

85. The Effects of ACTH and Adrenal Steroid Administration on Potassium Exchange in Human Erythrocytes. *David H. P. Streeten* (by invitation) and *Arthur K. Solomon* (by invitation) (introduced by *George W. Thorn*).

86. Effect of ACTH and Epinephrine upon Eosinophiles, Urinary Sodium, Potassium and 17-Ketosteroids in Adrenalectomized Patients. *William McK. Jefferies, Richard P. Levy* (by invitation) and *Luther W. Kelly* (by invitation).

87. Modification of Steroid Excretion in Cushing's Syndrome by a High Fat Diet. *Edna H. Sobel, Leland C. Clark, Jr.* (by invitation), *Solomon Papper* (by invitation), *Sol Sherry* (by invitation) and *Gwen Prior* (by invitation).

88. Effects of Injury and Dietary Factors on Adrenal Weight and Cholesterol Concentration. *Elaine P. Ralli, Mary E. Dumm* and *Herbert Gershberg.*

Steroids and Bioassay Methods

89. The Metabolism of β-Estradiol in Man. *Benjamin F. Stimmel* and *James A. May* (by invitation).

90. Quantitative Patterns of Urinary Corticosteroids. *Hildegard Wilson, Richard Fairbanks* (by invitation) and *Dominick Scialabba* (by invitation).

91. Studies on the Colorimetric Estimation of Estrogens. *Desmond Beall,* (by invitation) and *Gordon A. Grant.*

92. Bioassay of Adrenal Steroids in Blood. *Eugenia Rosemberg* (by invitation), *Jerome Cornfield* (by invitation), *Robert W. Bates* and *Evelyn Anderson.*

93. Steroid Studies: I. Metabolic Effects of Androstanol-17β,one-3 in Aged People. *Sidney Pearson, Jonas Weissberg* and *Thomas H. McGavack.*

94. Steroid Studies: II. Metabolism of Androstanol-17β,3-one. *Sidney Pearson* and *Thomas H. McGavack.*

95. A Micro Spectrophotometric Technique of Use in the Identification of C-19 Steroids. *Lytt I. Gardner.*

96. Quantitative Fluorometry of Estrogen Mixtures. *Joseph W. Goldzieher.*

Miscellaneous

97. An Effect in Vitro of Cortisone on Intracellular Potassium and Sodium Concentrations of Rabbit Leucocytes. *D. Laurence Wilson.*

98. Differences in the Effect of Cortisone, Compound F and ACTH on the Thyroid as Revealed by Microhistometry. *Boris Catz, Ihsan El-Rawi* (by invitation), *Ernest Geiger* (by invitation) and *Paul Starr.*

99. Critical Evaluation of Methimazole (1-Methyl-2-Mercaptoimidazole) in the Treatment of Hyperthyroidism. *Jacqueline Chevalley, Samuel Kenigsberg, Adolph Gerl* and *Thomas H. McGavack.*

100. Effects of Altered Thyroid Function upon the Metabolism of Endogenously Labeled Thyroid Hormone. *A. Albert.*

101. Comparative Effects of Thyroxine and Triiodothyronine in Thyroidectomized Rats. *Arthur E. Heming* and *Dorsey E. Holtkamp.*

102. Etiologic Factors in Sporadic Cretinism: An Analysis of 90 Cases. *Barton Childs* (by invitation), and *Lytt I. Gardner.*

103. Changes in Urinary Nitrogen Excretion During Stressful Life Experiences and their Relation to Thyroid Function. *Basil S. Hetzel, William W. Schottstaedt, Lawrence E. Hinkle, Jr. and Harold G. Wolff.* (introduced by *Willard O. Thompson.*)

104. Effects of *p*-Aminosalicylic Acid on Thyroid and Adrenal Function. *John R. Hogness* (by invitation), *Thomas Wong* (by invitation) and *Robert H. Williams.*

105. Cabbage and Turnips as a Cause of Endemic Goiter. *Isodor Greenwald.*

106. The Difficulties in the Clinical Management of Hermaphroditism. *Henry H. Turner, R. Palmer Howard, F. M. Lingenfelter* (by invitation) and *James P. Dewar* (by invitation).

107. The Effectiveness of Oral Cortisone in Sjogren's Syndrome. *Manuel M. Villaverde.*

108. Primordial Dwarfism with Pancytopenic Hypersplenism in Two Siblings. *Theodore C. Panos.*

109. The Hypothalamus in Cushing's Syndrome Due to Bilateral Adrenal Hyperplasia. *Thomas H. Maren* (introduced by *E. B. Astwood*).

110. The Incidence of Endocrine Dysfunction Following Postpartum Hemorrhage and/or Shock. *Norman G. Schneeberg, William H. Perloff* and *S. Leon Israel* (by invitation).

THE AMERICAN GOITER ASSOCIATION

The 1953 Annual Meeting of the American Goiter Association will be held May 7, 8 and 9, in the Drake Hotel, Chicago, Illinois.

THE AMERICAN GERIATRICS SOCIETY

The 10th Annual Meeting of the American Geriatrics Society will be held at the Hotel Commodore, New York City, May 28–30, 1953.

All members who plan to attend the meeting should reserve accommodations at the Hotel Commodore at an early date. The meeting is being held just before that of the American Medical Association and the hotels expect to be filled to capacity.

The program is probably the most interesting one that has been arranged for the Society up to the present time.

The Annual Dinner of the Society will be held on Friday evening, May 29, at 7:30 P.M., preceded by cocktails at 6:30 P.M. Wives and guests of members are welcome.

THE AMERICAN COLLEGE OF ALLERGISTS

The annual conclave of The American College of Allergists will be held at the Conrad Hilton Hotel in Chicago, April 24 to 29, 1953.

The first four days will be devoted to instruction under the tutelage of recognized authorities and the last three to a discussion and reporting of recent advances in the field of allergy by the investigators themselves. For detailed information write The American College of Allergists, La Salle Medical Building, Minneapolis 2, Minnesota.